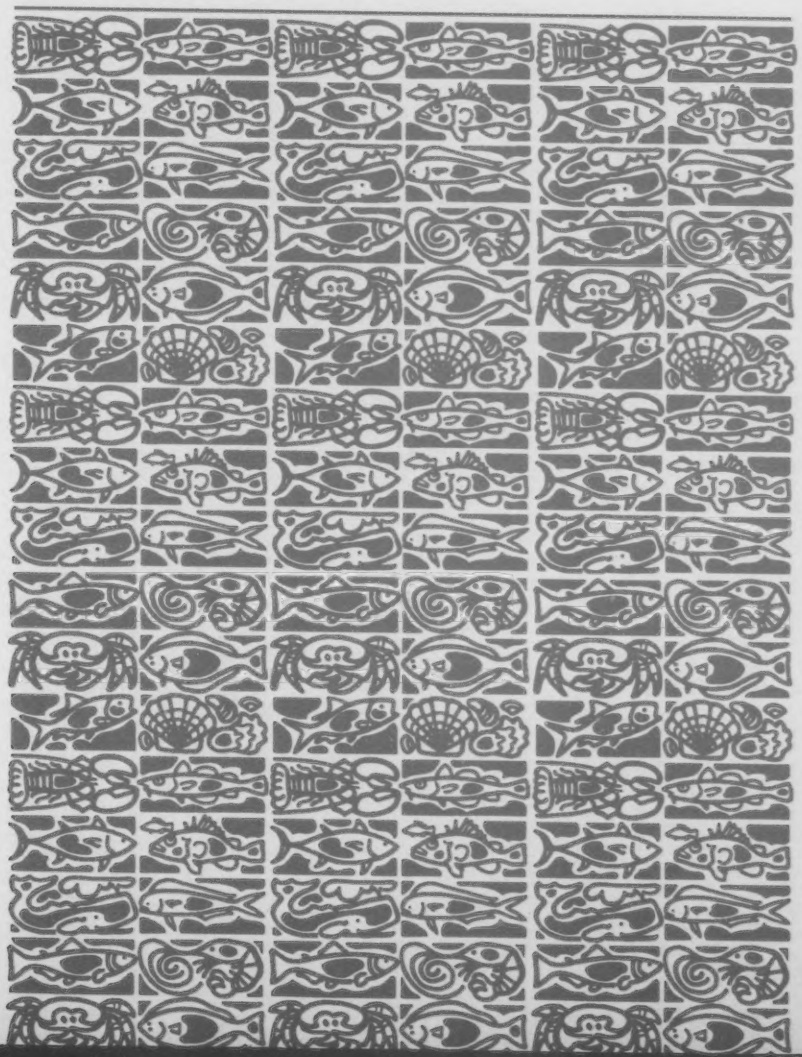
U.S. Department
of Commerce

Volume 102
Number 3
July 2004

Fishery Bulletin



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The *Fishery Bulletin* (ISSN 0090-0656) is published quarterly by the Scientific Publications Office, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, BIN C15700, Seattle, WA 98115-0070. Periodicals postage is paid at Seattle, WA, and at additional mailing offices. POSTMASTER: Send address changes for subscriptions to Fishery Bulletin, Superintendent of Documents, Attn.: Chief, Mail List Branch, Mail Stop SSOM, Washington, DC 20402-9373.

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The Secretary of Commerce has determined that the publication of this periodical is necessary according to law for the transaction of public business of this Department. Use of funds for printing of this periodical has been approved by the Director of the Office of Management and Budget.

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402. Subscription price per year: $55.00 domestic and $68.75 foreign. Cost per single issue: $28.00 domestic and $35.00 foreign. **See back for order form.**

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U.S. Department of Commerce
Seattle, Washington

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Testicular development in migrant and spawning bluefin tuna (*Thunnus thynnus* (L.)) from the eastern Atlantic and Mediterranean

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Abstract—Testis histological structure was studied in bluefin tuna (*Thunnus thynnus*) from the eastern Atlantic and Mediterranean during the reproductive season (from late April to early June). Testicular maturation was investigated by comparing samples from bluefin tuna caught on their eastward reproductive migration off Barbate (Strait of Gibraltar area) with samples of bluefin tuna fished in spawning grounds around the Balearic Islands. Histological evaluations of cross sections showed that the testis consists of two structurally different regions, an outer proliferative region where germ cells develop synchronously in cysts, and a central region made up of a well-developed system of ducts that convey the spermatozoa produced in the proliferative region to the main sperm duct. Ultrastructural features of the different stages of the male germ cell line are very similar to those described in other teleost species. The bluefin tuna testis is of the unrestricted spermatogonial testicular type, where primary spermatogonia are present all along the germinative portion of the lobules. All stages of spermatogenesis were present in the gonad tissue of migrant and spawning bluefin tuna, although spermatids were more abundant in spawning fish. The testis size was found to increase by a factor of four (on average) during migration to the Mediterranean spawning grounds, whereas the fat bodies (mesenteric lipid stores associated with the gonads) became reduced to half their weight, and the liver mass did not change significantly with sexual maturation. Linear regression analysis of the pooled data of migrant and spawning bluefin tuna revealed a significant negative correlation between the gonad index (I_G) and the fat tissue index (I_F), and a weaker positive correlation between the gonad index (I_G) and the liver index (I_L). Our analyses indicate that the liver does not play a significant role in the storage of lipids and that mesenteric lipid reserves constitute an important energy source for gametogenesis in bluefin tuna.

Manuscript submitted 27 April 2003 to Scientific Editor's Office.

Manuscript approved for publication 25 March 2004 by the Scientific Editor.

Fish. Bull. 102:407–417 (2004).

The Atlantic northern bluefin tuna (*Thunnus thynnus thynnus* (L.)), is one of the most commercially valuable wild animals in the world. In the last two decades this species has been subject to intense over-fishing, which has caused a decline in both the eastern and western populations because of lowered recruitment (Mather et al., 1995; Sissenwine et al., 1998). The bluefin tunas (*T. thynnus* and *T. maccoyii*) are unique among tuna species in that they live mainly in cold waters and move into warmer waters to spawn (Olson, 1980; Lee, 1998; Schaefer, 2001); therefore the migratory pattern of these species depends substantially on reproduction. The eastern stock of Atlantic bluefin tuna spawns from June through August in the Mediterranean Sea, where natural conditions are apparently optimal for the survival of offspring. From late April to mid June, bluefin tuna breeding stocks migrate from the North Atlantic to spawning grounds in the Mediterranean (Mather et al., 1995; Ravier and Fromentin, 2001). A good understanding of the reproductive parameters (especially sexual maturation, fecundity, and spawning) of tunas is of paramount importance for population dynamics studies and the management of fisheries that target tunas. Nevertheless, "a very limited amount of scientifically useful information is available on the reproductive biology for most tunas" (Schaefer, 2001). Recent work has increased our knowledge on the reproductive biology of female *Thunnus thynnus* in the eastern Atlantic and the Mediterranean (Susca et al., 2000, 2001a, 2001b; Hattour and Macías, 2002; Medina et al., 2002; Mourente et al., 2002), but many questions remain still to be answered regarding male reproductive activity in this and other tuna species.

Histological examination of gonads is a useful tool for assessing the maturity state of fish. However, very few light-microscopy studies have been published on bluefin tuna and no ultrastructural studies of reproductive organs are yet available. The male reproductive cycle of *T. thynnus* has been characterized histologically by Santamaria et al. (2003), and Ratty et al. (1990) and Schaefer (1996, 1998) have reported valuable histological descriptions on male and female gonads of the Pacific albacore (*Thunnus alalunga*) and the yellowfin tuna (*Thunnus albacares*), respectively. In this article we report biometric and histological data on male *T. thynnus* caught during their reproductive migration and spawning period in order to provide further information on the biological aspects of reproduction for this species.

Materials and methods

Samples and condition indices

During the eastward migration, 62 adult male bluefin tuna weighing between 71 and 273 kg (mean 195.17 kg) were obtained from the trap fishery in the area of the Strait of Gibraltar (Barbate, Cádiz, southwestern Spain) from late April to early June 1999, 2000, and 2001. Thirty-four mature males, weighing between 19 and 349 kg (mean 115.11 kg), were sampled in June–July 1999–2001 from the purse-seine fleet operating in the Mediterranean spawning grounds of bluefin tuna off the Balearic Islands. Whenever possible, the total body weight (W) was recorded to the nearest kg. When individual body weights were not available, W was estimated from the fork length (L_F) measurements (recorded to the nearest cm), according to the formula: $W = 0.000019 \times L_F^3$ (Table VIII in Rodríguez-Roda, 1964). Following dissection, the liver, testes, and the fat bodies associated with the gonads were removed and weighed to the nearest g. The condition of the fish was assessed by three different indices. The gonad index (gonadosomatic index) (I_G) is indicative of the maturation state and was calculated as: $I_G = (W_G / W) \times 100$, where W_G = gonad weight. The liver index (hepatosomatic index) (I_L) and fat-body index (I_F) were calculated as $I_L = (W_L / W) \times 100$, and $I_F = (W_F / W) \times 100$ (where W_L and W_F represent liver and fat-body weights), respectively, and are considered as good indicators of the metabolic condition and energy reserves of the fish. All measurements are expressed as means ±SD.

Histology

For light microscopy, tissue samples from the central part of the testes were fixed for 48–96 hours in 10% formalin in phosphate buffer, 0.1 M, pH 7.2. After dehydration in ascending concentrations of ethanol, a part of each sample was embedded in paraffin wax and the remainder was embedded in plastic medium (2-hydroxyethyl-methacrylate). Paraffin sections (6 μm thick) were stained with haematoxylin-eosin, and plastic sections (3 μm thick) were stained with toluidine blue. These were examined and photographed on a Leitz DMR BE light microscope.

For electron microscopy, small fragments of testis were fixed for 3–4 hours in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer (pH 7.2). Following two 30-min washes in cacodylate buffer, they were postfixed for 1 hour at 4°C in cacodylate-buffered 1% osmium tetroxide, rinsed several times in buffer, dehydrated in ascending concentrations of acetone, and embedded in epoxy resin (either Epon 812 or Spurr). Thin sections (~80 nm thick) were picked up on copper grids, stained with uranyl acetate and lead citrate, and examined in a Jeol 1200 EX transmission electron microscope. Approximate dimensions provided for germ cells are measurements (means ±SD) of the largest cell diameters on electron micrographs.

Statistical analysis

The bluefin tuna specimens used in this study showed considerable variability in size, especially those caught by purse seine in Balearic waters, where weight ranged between 12 and 349 kg. The purse-seine fishery is, in fact, much less size-selective than are traps, which seldom catch small bluefin tuna (Rodríguez-Roda, 1964; Mather et al., 1995). Analysis of covariance (ANCOVA), with body weight as covariate, was used as the most suitable method (García-Berthou, 2001) to test interannual differences in the weight of the organs within the two sampling sites. ANCOVA was likewise applied to compare the weights of the three organs between both areas. All data were previously log-transformed to meet the prerequisites of normality and homoscedasticity (Zar, 1996). Linear least-squares regression analyses were performed to test possible correlations between I_G and the two other indices (I_L and I_F) by using the pooled data of Barbate and the Balearic Islands. In the regression between I_G and I_F, the Balearic samples corresponding to year 2001 were excluded because the reduced fat-body size (adipose tissue was almost nonexistent in the mesentery) of these small bluefin tuna did not permit an accurate weight measurements on board. The values of the indices were arcsine-transformed prior to the statistical analysis (Zar, 1996). A P-value ≤0.05 was considered statistically significant for all tests.

Results

Condition indices

ANCOVA did not reveal significant interannual differences in gonad, liver, and fat-body weight in the samples of Barbate as well as in those of the Balearic Islands. In contrast, a strongly significant difference in testicular size ($P<0.0001$) was found in comparing data of maturing bluefin tuna from Barbate (migrant tuna) with fully mature fish from the Balearic Islands (spawning fish). In fact, as shown in Figure 1, the average I_G was more than fourfold higher in the Balearic Islands than it was in Barbate (4.81 ±1.77 vs. 1.12 ±0.57). This finding may indicate a noticeable increase in sperm production during reproductive migration to the Mediterranean spawning grounds. Significant differences between maturing and spawning tuna were also found in fat-body weight, the volume of which dropped to about half by spawning time. Thus, I_F fell from 0.36 ±0.24 in migrating fish to 0.16 ±0.12 in spawning fish (see Fig. 1). The liver mass, however, did not differ significantly ($P=0.31$) between the two samples.

Figure 2 illustrates linear regression analysis between I_G and I_L, and between I_G and I_F. A significant negative correlation ($r^2=0.34$; $P<0.0001$) was found between I_G and I_F, indicating that the amount of mesenteric fat tissue decreases as the gonad matures. In contrast, there was a positive, though somewhat weak, correlation

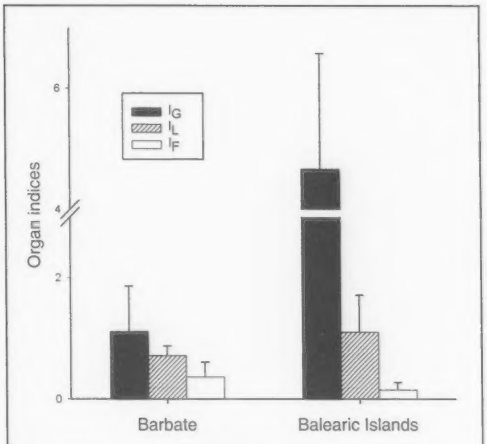


Figure 1

Differences in gonad index (I_G), liver index (I_L), and fat-body index (I_F) (means ±SD) between male bluefin tuna (*Thunnus thynnus*) from Barbate and male tuna from the Balearic Islands.

(r^2=0.21; P<0.0001) between I_G and I_L, which suggests a slight growth of the liver with sexual maturation.

Histology

The testes of *Thunnus thynnus* are paired, elongate organs that appear attached to the dorsal body wall by a mesentery. The fat body, which is closely associated with the gonad, consists of a variable amount of adipose tissue. The testis is composed of a dense array of lobules converging on the main sperm duct (vas deferens) and terminating blindly beneath the tunica albuginea at the periphery (Fig. 3, A and B). Two distinct zones can be distinguished in cross sections of the testes (Fig. 3A). At the outer region, the seminiferous lobules have a thick wall formed by the germinal epithelium, where germ cells develop in association with Sertoli cells; the lumina of the lobules are filled with spermatozoa that have been released after completion of the spermiogenetic process (Fig. 3, B and C). As a result of the release of mature sperm from spermatocysts into the lobule lumina, the germinal epithelium becomes discontinuous (Fig. 3B). The transition from the outer to the central region of the testis is marked by an abrupt change in the configuration of the testicular lobules, which lose the germinal epithelium and become ducts where lobule function has shifted from sperm production to sperm storage (Fig. 3C). Thus, the only sex cells that are found in the central part of the testis are mature spermatozoa, which fill the swollen lumina of the lobules. In this zone the testis ducts constitute an intricate network of channels that convey the spermatozoa produced in the proliferative region to the main sperm duct (Fig. 3, A and D), which is thick walled and located in the center of the testis (Fig. 3D).

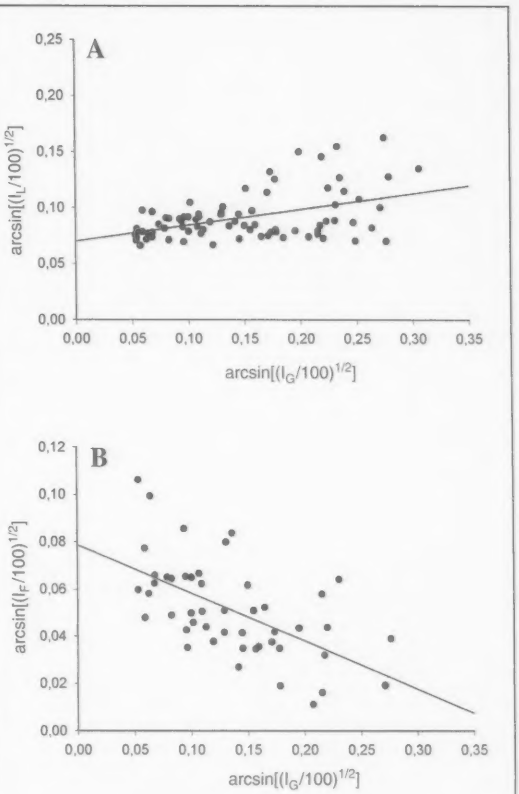


Figure 2

Linear regression between gonad index (I_G) and liver index (I_L) (**A**), and between gonad index (I_G) and fat body index (I_F) (**B**) (data were pooled from the two areas). In B, samples of bluefin tuna (*Thunnus thynnus*) collected off the Balearic Islands in 2001 were excluded.

The gametes develop in groups of isogenic cells called germinal cysts or spermatocysts, where the process of differentiation is synchronous (Fig. 4). Primary spermatogonia are large, single cells (Fig. 4A) that are distributed all along the germinal epithelium, as is characteristic of the teleost unrestricted testicular type. Spermatogonia B resulting from successive mitoses of spermatogonia A are found in small groups, whereas spermatocytes and spermatids are grouped within larger spermatocysts (Figs. 3, B and C, 4). The cysts containing late spermatids and spermatozoa, prior to spermiation, display a particular alveolar appearance due to the orientation of the spermatid heads facing the lobule walls and the bundles of flagella directed toward the seminiferous lobule lumen (Fig. 4, A, C, and D).

Active spermatogenesis was observed to occur both in migrant bluefin tuna from the Strait of Gibraltar (Fig. 4, A and B) and spawning fish from the Mediterranean (Fig. 4, C and D). In both cases, all stages of the male

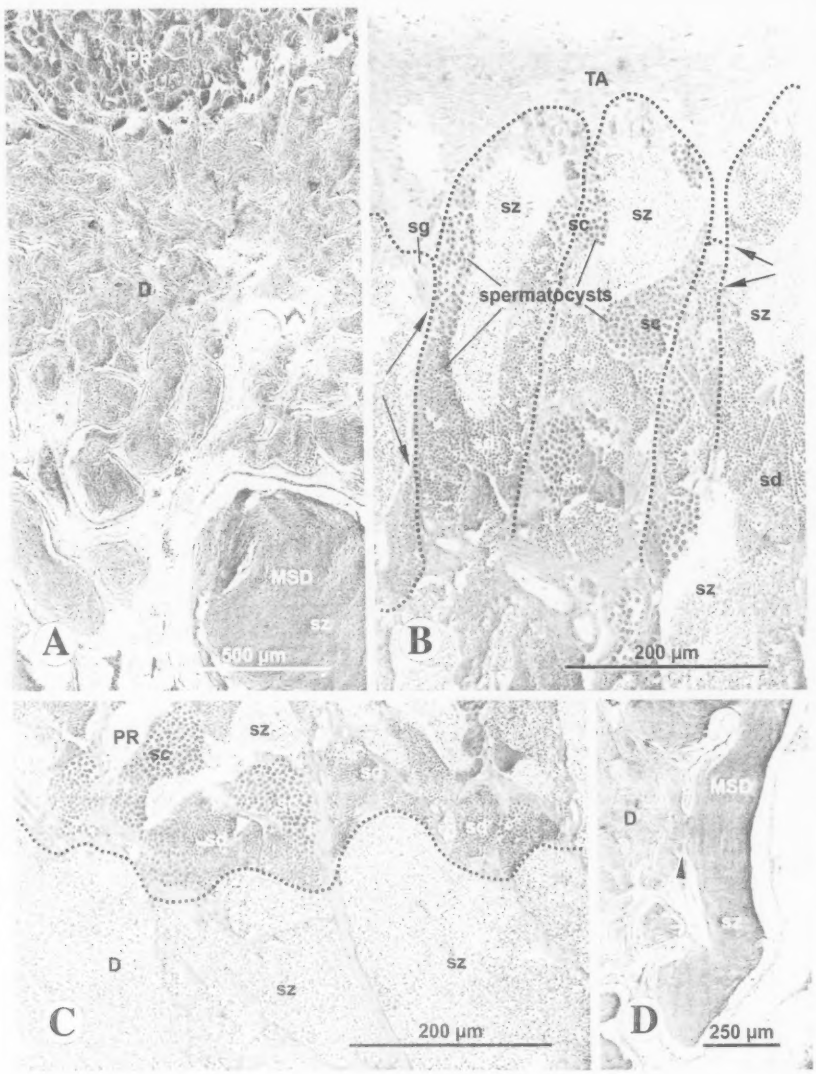


Figure 3

Light micrographs depicting the histological organization of the bluefin tuna (*Thunnus thynnus*) testis. (**A**) Transverse section showing the outer proliferative region (PR) of the testis and the inner region, which includes the testis duct system (D) and the main sperm duct (MSD). (**B**) Peripheral zone of the testis where the distal ends of some tubules (dotted lines) terminate beneath the tunica albuginea (TA). (**C**) Transition (dotted line) between the outer region (PR) of the testis, where the lobules contain developing germinal cysts, and the inner region (D), whose lobule walls enclose only mature spermatozoa. (**D**) Main sperm duct (MSD) filled with a compact mass of spermatozoa that are incorporated (arrowhead) from the testis ducts (D). Arrows = discontinuities in the germinal epithelium; sc = spermatocytes; sd = spermatids; sg = spermatogonia; sz = spermatozoa. All samples are from Barbate (A and D, paraffin-embedded sections stained with haematoxylin-eosin; B and C, toluidine-blue–stained plastic-embedded sections).

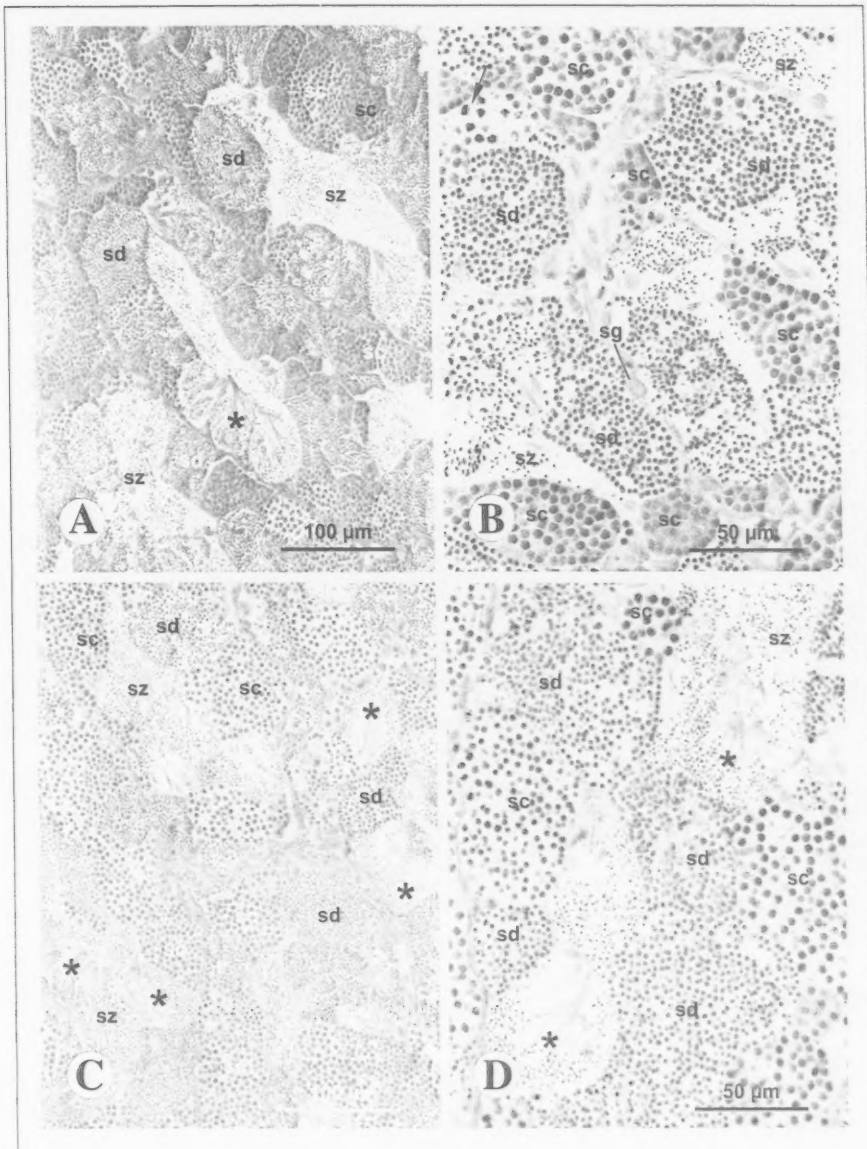
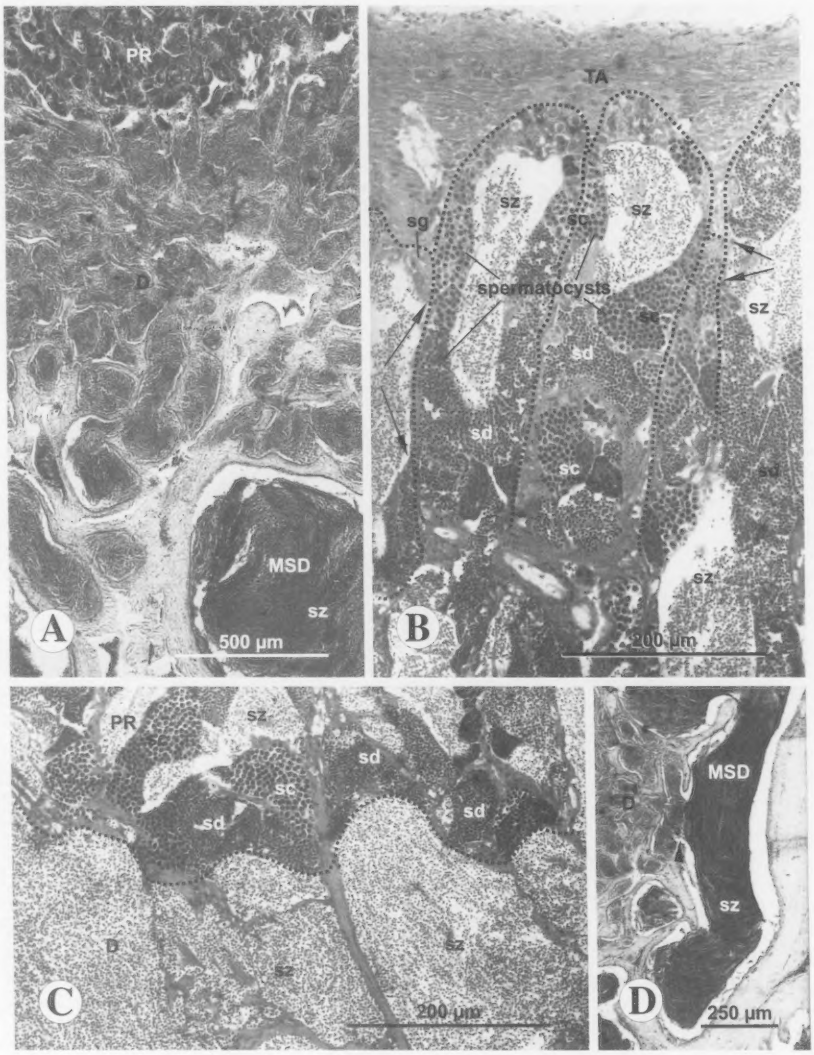


Figure 4

Spermatocysts in the testis proliferative region of bluefin tuna (*Thunnus thynnus*) from Barbate (**A** and **B**) and the Balearic Islands (**C** and **D**). All stages of spermatogenesis are present in both cases, although spermatid cysts containing late spermatids and spermatozoa (asterisks) are somewhat more abundant in specimens from the Balearic Islands. Arrow = dividing spermatocytes; sc = spermatocytes; sd = spermatids; sg = primary spermatogonion; sz = spermatozoa. Plastic-embedded sections (A–D) were stained with toluidine blue.

germ cell line were present in the gonads. In addition, large amounts of spermatozoa had accumulated in the central system of ducts and in the main sperm duct, both of which appear to function as reservoirs of sperm. In specimens from Barbate, spermatocytes and spermatids were abundant (Fig. 4B), whereas in most tuna collected in the Balearic area spermatids predominated over spermatocytes. Cysts containing late spermatids and spermatozoa were particularly common (Fig. 4, C and D).



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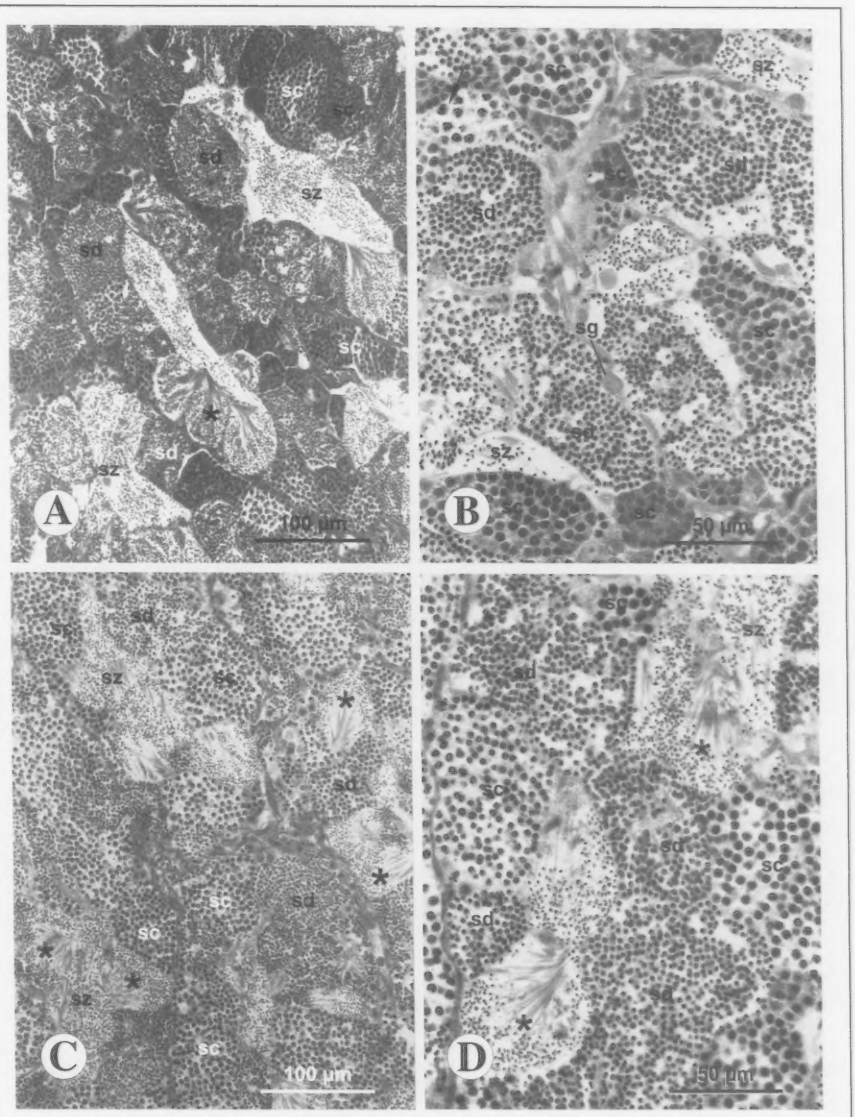

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Spermatocysts in the testis proliferative region of bluefin tuna (*Thunnus thynnus*) from Barbate (**A** and **B**) and the Balearic Islands (**C** and **D**). All stages of spermatogenesis are present in both cases, although spermatid cysts containing late spermatids and spermatozoa (asterisks) are somewhat more abundant in specimens from the Balearic Islands. Arrow = dividing spermatocytes; sc = spermatocytes; sd = spermatids; sg = primary spermatogonion; sz = spermatozoa. Plastic-embedded sections (A–D) were stained with toluidine blue.

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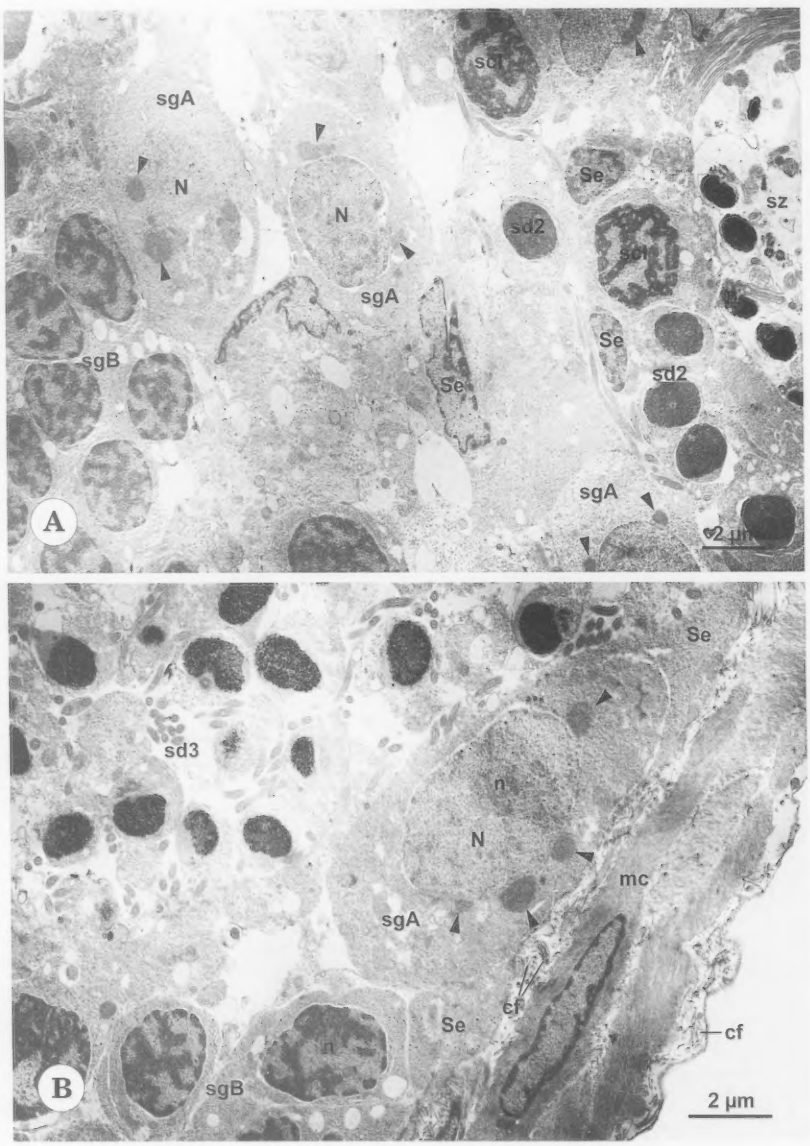

Figure 5

Electron micrographs of testicular tissue from bluefin tuna (*Thunnus thynnus*). (**A** and **B**) overviews displaying several spermatocyst types, including single primary spermatogonia (sgA), and clusters of spermatogonia B (sgB), primary spermatocytes (scI), mid spermatids (sd2), late spermatids (sd3), and spermatozoa (sz). The germ cells are surrounded by Sertoli cells (Se). Arrowheads = perinuclear bodies ("nuage"); N = nucleus; n = nucleolus; cf = collagen fibers; mc = myoid cell.

Ultrastructure

Primary spermatogonia are large, ovoid cells (8.55 ±1.07 µm) whose nucleus (>5 µm in its largest diameter) shows diffuse chromatin and a single central nucleolus. The cytoplasm contains free ribosomes, a few mitochondria, endoplasmic reticulum cisternae, and several masses

of electron-dense perinuclear material ("nuages") that indicate nucleocytoplasmic transport (Fig. 5, A and B). Such chromatoid bodies persist throughout spermatogenesis until the spermatid stage, but their size and number is far higher in primary spermatogonia. Spermatogonia B are grouped in clusters of a few cells. They are perceptibly smaller (6.75 ±0.37 µm) than spermatogonia A and their nucleus contains patchy chromatin (Fig. 5, A and B).

Spermatocytes form clusters in which the cells are interconnected by cytoplasmic bridges. Primary spermatocytes (4.84 ±0.45 µm) show a heterochromatic nucleus (~3.5 µm in diameter) that varies in appearance depending on the prophase-I stage. The cytoplasm contains free ribosomes (mostly polysomes), mitochondria, clear vesicles, and the diplosome (Figs. 5A, 6A). Synaptonemal complexes are clearly recognizable at pachytene (Fig. 6A). Secondary spermatocytes are apparently short-lived cells because they are rare in histological samples—a finding that suggests that the second meiotic division is triggered shortly after completion of the first division. Spermatocytes II are difficult to distinguish morphologically from early spermatids, although they are slightly larger (3.31 ±0.47 µm). The cytoplasm is more reduced than in spermatocytes I and the nucleus shows diffuse chromatin forming moderately electron-dense patches (Fig. 6B).

During spermiogenesis, the spermatid nucleus changes in shape and decreases in volume as the chromatin condenses. In early spermatids (2.39 ±0.28 µm) the spherical nucleus shows a dense chromatin with some electron-lucent areas (Fig. 6C). Then the chromatin becomes more homogeneous in mid spermatids (2.56 ±0.21 µm) (Figs. 5A, 6D), and eventually in late spermatids (1.81 ±0.32 µm) condenses into a coarse granular pattern, whereas the nucleus assumes an ovoid shape and forms a basal indentation over the proximal segment of the axoneme (Fig. 6E). Cytoplasmic changes involve elongation of the flagellum, reduction of the cytoplasmic mass, and coalescence of the mitochondria into a few large spherical units located around the proximal portion of the axoneme. Rotation of the nucleus does not take place during spermiogenesis, therefore the flagellum axis remains parallel to the base of the nucleus and the spermatozoon shows the typical ultrastructure of teleostean type-II sperm (Fig. 6F).

Discussion

Histologically, the bluefin tuna testis is of the unrestricted spermatogonial testicular type found in most teleosts, where spermatogonia occur along the greater part of the testicular tubules. In the restricted spermatogonial testicular type of the atheriniforms, on the other hand, the spermatogonia are confined to the distal end of the tubules, and spermatogenesis proceeds as the germ cells approach the efferent ducts (Grier et al., 1980; Grier, 1981). Efferent ducts are generally absent in unrestricted spermatogonial testes, so that germinal cysts form along the testicular tubule length (Grier et al., 1980; Grier, 1981; Lahnsteiner et al., 1994). However, in maturing and spawning bluefin tuna a well-developed network of ducts collects the sperm produced by the germinal epithelium and voids them into the main sperm duct. The central ducts of the testis are continuous with the proliferative segment of the testicular lobules, which lose the germinal epithelium in the innermost region of the testis and function as sperm storage structures. This process has been documented in the common snook (*Centropomus undecimalis*) (Grier and Taylor, 1998), the cobia (*Rachycentrum canadum*) (Brown-Peterson et al., 2002), and the swamp eel (*Synbranchus marmoratus*) (Lo Nostro et al., 2003). Grier et al. (1980) showed that in the atheriniform *Fundulus grandis* the efferent duct wall cells derive from Sertoli cells. A system of efferent ducts has been described in other species of teleosts possessing testes of the unrestricted spermatogonial type (Rasotto and Sadovy, 1995; Manni and Rasotto, 1997). As has been shown in other species of the genus (Ratty et al., 1990; Schaefer, 1996; 1998), the main sperm duct of *T. thynnus* has a thick wall and is located near the center of the testis, whereas in many other teleosts the main duct is dorsal (Grier et al., 1980).

Ultrastructural features of bluefin tuna spermatogenesis are comparable to those described extensively in teleosts (for examples of recent literature see Gwo and Gwo, 1993; Stoumboudi and Abraham, 1996; Quagio-Grassiotto et al., 2001; Huang et al., 2002; Koulish et al., 2002; Fishelson, 2003). The primary spermatogonia are the largest male germ cells and exhibit several conspicuous perinuclear ("nuage") bodies. After several divisions they give rise to cysts of secondary spermatogonia that enter meiosis to produce successively primary and secondary spermatocytes. Primary spermatocytes are abundant, particularly at the pachytene phase, and are therefore thought to be of long duration. In contrast, the spermatocyte-II stage is thought to be the shortest spermatogenetic step, because, as occurs in teleosts in general, it is the least frequent in histological samples. Spermiogenesis develops without the occurrence of rotation of the spermatid nucleus, resulting in a teleostean type-II spermatozoon (Mattei, 1970), in which the flagellar axis lies tangential to the nucleus instead of being inserted perpendicular to its base (Abascal et al., 2002).

Santamaria et al. (2003) divided the testicular cycle of *T. thynnus* caught in Mediterranean waters from February to September into five periods. Those developmental stages are similar to stages 2–6 classified by Grier (1981) for a generalized teleost annual reproductive cycle. Most probably, stage 1 (spermatogonial proliferation) occurs in Mediterranean bluefin tuna between October and January. More recently, annual histological changes in the germinal epithelium have been used to identify five distinct reproductive classes in males of several teleost species (Grier and Taylor, 1998; Taylor et al., 1998; Brown-Peterson et al., 2002; Lo Nostro et al., 2003). It is assumed that the most advanced maturation classes in males are characterized by the presence of a discontinuous germinal epithelium. According to this criterion, all

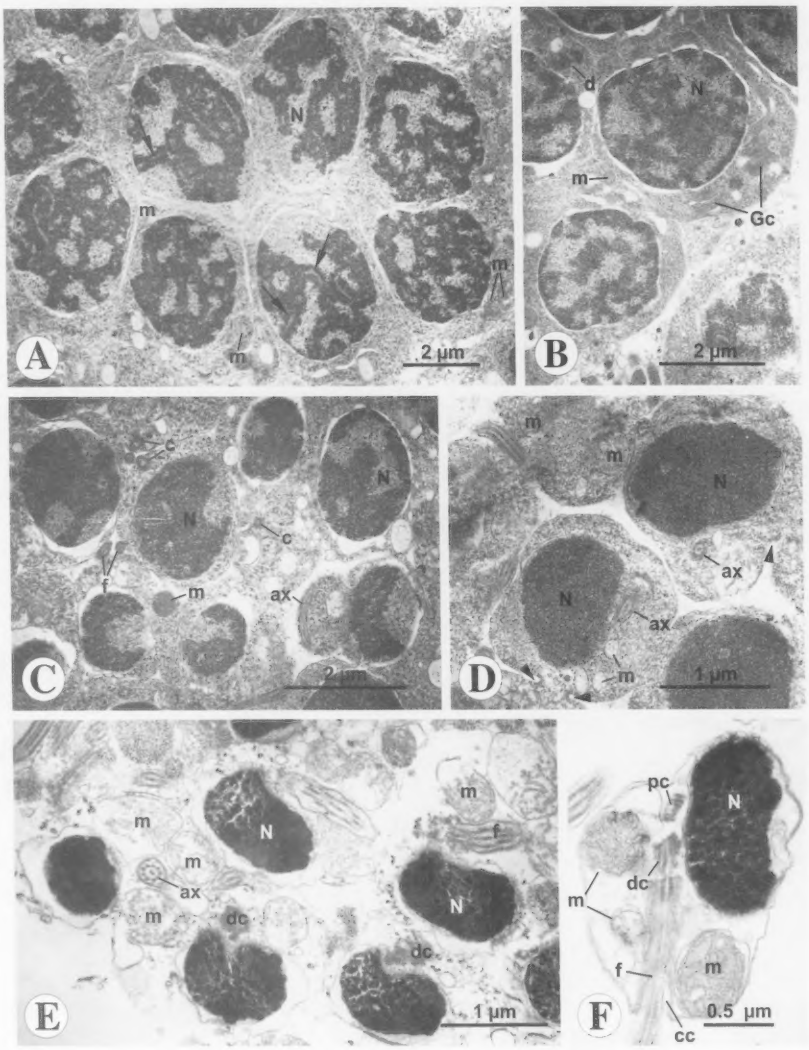

Figure 6

Electron micrographs of spermatocytes I (**A**), spermatocytes II (**B**), early spermatids (**C**), mid spermatids (**D**), late spermatids (**E**), and spermatozoon (**F**) from bluefin tuna (*Thunnus thynnus*). Arrows = synaptonemal complexes; arrowheads = cytoplasmic bridges between spermatids; ax = axoneme; c = centriole; cc = cytoplasmic canal; d = diplosome; dc = distal centriole; f = flagellum; Gc = Golgi complex; m = mitochondria; N = nucleus; pc = proximal centriole.

of the samples examined in the present study correspond to the mid- and late-maturation stages proposed by Grier and Taylor (1998), and Taylor et al. (1998). Testes at these stages become storage organs that are filled with sperm. The present study encompasses only a short period of the reproductive cycle, which comprises final gonad maturation. However, descriptions of the testicular histology throughout the annual cycle (Santamaria et al., 2003) appear to indicate that different maturation classes might be defined in the bluefin tuna based on histological examination of the germinal epithelium (see Taylor et al., 1998; Brown-Peterson et al., 2002).

Final sexual maturation involves a considerable increase in testis size, but no apparent remarkable histological changes, with the exception of a slightly higher frequency of the most advanced stages of spermatogen-

esis in fully mature bluefin tuna. The different testicular development of maturing and spawning tuna is reflected by their respective average I_G, which was fourfold higher in spawning fish. An equivalent gonad growth was found in the females collected in the same samplings (Medina et al., 2002), indicating a spatiotemporal parallelism in the gonad maturation cycle and a good synchronization of the reproductive peak in the two sexes. The maturation schedule differs between the two sexes, however, in that males are capable of generating mature spermatozoa while still on migration, whereas females do not appear to develop fully mature oocytes until they have reached the spawning grounds (Medina et al., 2002). Therefore, even though mature spermatozoa can be found in testicular ducts during prolonged periods throughout the reproductive cycle, it is unlikely that males are actually capable of spawning out of reproductive season.

The seasonal I_G profile of the bluefin tuna appears to be similar to that of the pelagic, highly migratory perciform *Rachycentron canadum* (Brown-Peterson et al., 2002), and the swamp eel (*Synbranchus marmoratus*) (Lo Nostro et al., 2003), in which peak I_G values occur when the reproductive activity is at a maximum. A different situation has been reported in the common snook (Taylor et al., 1998), where the highest I_G levels correspond with the mid maturation class and decrease during the latter part of the reproductive season. The biological significance of these different I_G profiles in terms of reproductive strategies is yet unknown because a very limited number of species have been examined so far.

Because spermatozoa are by far the most abundant cells in mature testes, the gonad weight becomes a good indicator of the quantity of sperm produced by a fish (Billard, 1986). Therefore, the significant increase in I_G that occurred between samplings off Barbate and the Balearic Islands would indicate that, during migration, bluefin tuna can raise several times the volume of sperm accumulated in the testes. The apparently high spermatogenetic activity observed in bluefin tuna caught on the spawning grounds suggests that bluefin tuna have the ability to regenerate testicular sperm stores. Continuous sperm production could be important because external fertilization requires the release of large amounts of sperm to ensure successful fertilization of eggs, especially when egg size is small. In addition, it should be noted that tunas spawn multiple times (June, 1953; Yuen, 1955; Buñag, 1956; Otsu and Uchida, 1959; Baglin, 1982; Stéquert and Ramcharrun, 1995) and can spawn almost daily throughout the reproductive season (Hunter et al., 1986; McPherson, 1991; Schaefer, 1996, 1998, 2001; Farley and Davis, 1998; Medina et al., 2002).

From histological examination of the sperm ducts, and based on the amount of sperm present and the staining of the epithelium, Schaefer (1998) proposed a spawning interval of 1.03 days for spawning male *Thunnus albacares* throughout the eastern Pacific Ocean. The spawning rate estimated for reproductively active females with the postovulatory-follicle method was 1.19 days (Schaefer, 1998), which coincides with the spawning interval estimated for female *T. thynnus* around the Balearic Islands (Medina et al., 2002). Unfortunately, we could not make a reliable estimation of the male spawning interval in our samples. Two possible reasons may account for this failure. One reason is that many of the samples of gonadal tissue did not include the main sperm duct. On the other hand, no clear evidence of spawning was identified by histological examination of those specimens processed that had sperm ducts. A plausible explanation for this fact is that recent sperm release can be detected only within 12 hours after the spawning event (Schaefer, 1996); hence for male spawning to be detected the fish would have to be sampled in a narrow range of times following spawning, which Schaefer (1996) established between 00.01 and 12.00 hours after spawning for *Thunnus albacares*. It would be worth conducting further research on bluefin tuna at their spawning grounds, by attempting to cover a broad range of sampling times in order to ensure collection of specimens shortly after gamete release. In this way, useful information would be obtained on such reproductive parameters as spawning schedules, fecundity, and the energy cost of spawning, which are essential for ecological assessments of the reproductive potential.

It is noteworthy that male tuna, as small as 20 kg in weight (~100 cm L_F), were caught on the spawning grounds in our study. They had gonad indices over 5% and histological features indicative of full maturity. These observations indicate that the eastern stock of Atlantic northern bluefin tuna can reach maturity at age 3 years and thus support conclusions of previous studies (Rodríguez-Roda, 1967; Hattour and Macías, 2002; Susca et al., 2001a, 2001b; Medina et al., 2002); western bluefin tuna, on the other hand, mature at an older age, which has been estimated at 6 years (Baglin, 1982).

Prior to sexual maturation, marine fish generally accumulate large lipid deposits, primarily triacylglycerols, which are subsequently mobilized to support gonad development and spawning migration (Bell, 1998). The major lipid storage sites are the mesenteric tissue, muscle, liver, and subdermal fat layers (Ackman, 1980). In bluefin tuna the liver does not appear to play an important role in lipid storage but is mainly involved in processing fatty acids mobilized from other bodily sources (Mourente et al., 2002). This metabolic pattern is consistent with our observations of weight modifications for liver and fat body from maturation through the spawning period. Although I_L increases only slightly with sexual maturation, I_F undergoes a marked decrease at the time of maximum gonad development. Thus, the regression analysis of the relationship between I_G and I_F shows a significant negative correlation, which reveals a depletion of mesenteric fat stores as the testes grow. The occurrence of a similar situation in females (Medina et al., 2002; Mourente et al., 2002) and in male and female *Thunnus alalunga* (Ratty et al., 1990) has led to the conclusion that fat-body lipid reserves provide an important energy source for gametogenesis in tunas.

Acknowledgments

This study has been funded by the Spanish government and the European Union (projects 1FD1997-0880-C05-04 and Q5RS-2002-01355). The authors wish to thank two anonymous reviewers for helpful recommendations. We also thank Pesquerías de Almadraba, S. J. and Ginés J. Méndez Alcalá (G. Méndez España, S. L.) for co-operation and assistance. The invaluable technical assistance of Agustín Santos and José Luis Rivero is greatly appreciated. G. Mourente lent useful help in our discussion on lipids.

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Abstract—From 1995 to 1998, we collected female black rockfish (*Sebastes melanops*) off Oregon in order to describe their basic reproductive life history and determine age-specific fecundity and temporal patterns in parturition. Female black rockfish had a 50% probability of being mature at 394 mm fork length and 7.5 years-of-age. The proportion of mature fish age 10 or older significantly decreased each year of this study, from 0.511 in 1996 to 0.145 in 1998. Parturition occurred between mid-January and mid-March, and peaked in February. We observed a trend of older females extruding larvae earlier in the spawning season and of younger fish primarily responsible for larval production during the later part of the season. There were differences in absolute fecundity at age between female black rockfish with prefertilization oocytes and female black rockfish with fertilized eggs; fertilized-egg fecundity estimates were considered superior. The likelihood of yolked oocytes reaching the developing embryo stage increased with maternal age. Absolute fecundity estimates (based on fertilized eggs) ranged from 299,302 embryos for a 6-year-old female to 948,152 embryos for a 16-year-old female. Relative fecundity (based on fertilized eggs) increased with age from 374 eggs/g for fish age 6 to 549 eggs/g for fish age 16.

Manuscript submitted 13 March 2003 to Scientific Editor's Office.

Manuscript approved for publication 30 March 2004 by the Scientific Editor.

Fish. Bull. 102:418–429 (2004).

Maturity, ovarian cycle, fecundity, and age-specific parturition of black rockfish (*Sebastes melanops*)

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Many fish species in the North Pacific have a long reproductively active life span, which increases the likelihood of producing offspring during periods of favorable environmental conditions. This bet hedging reproductive strategy reduces the impact of environmental variation on reproductive success (Goodman, 1984; Leaman and Beamish, 1984; Schultz, 1989). In species with age-structured spawning schedules, a broad age distribution will maximize the length of the spawning season. The more protracted the reproductive period, the greater the likelihood that some spawning will occur during conditions favorable for larval survival (Lambert, 1990). Age-related differences in the timing of spawning have been observed in many fishes; usually larger, older fish spawn earlier (Simpson, 1959; Bagenal, 1971; Berkeley and Houde, 1978; Shepherd and Grimes, 1984; Lambert, 1987), but in some cases younger fish spawn earlier in the season (Hutchings and Myers, 1993).

Age truncation, an inevitable result of fishing, can increase recruitment variability by reducing the length of the spawning season or by selectively removing older, more fit individuals from the population. Factors that might affect individual reproductive success include the number of eggs produced, the quality of eggs (e.g., yolk or oil globule volume), and the size or health of eggs and larvae. Off the coast of Oregon, widow rockfish (*Sebastes entomelas*) have exhibited increased absolute fecundity, and more importantly have increased relative fecundity, with age (Boehlert et al., 1982). Individual populations of shortbelly rockfish (*Sebastes jordani*), have been found to produce larvae with differing lipid and protein compositions and consequently potentially differing rates of survival (MacFarlane and Norton, 1999). Zastrow et al. (1989) reported that striped bass eggs stripped from wild fish increase in quality with maternal age due to increased amounts of proteins and lipids, although relative concentrations remain unchanged.

Black rockfish (*Sebastes melanops*), like most other rockfish, are long-lived, moderately fecund livebearers with long reproductive life spans. Although their longevity and low rate of natural mortality is presumed to be an adaptation to allow successful reproduction over their lifespan despite long periods between favorable environmental conditions, it also makes them more susceptible to overexploitation. The objective of our research presented in the present article is twofold. First, we describe the basic reproductive life history of black rockfish, with an emphasis on the ovarian developmental cycle and maturity schedule. Second, we investigate age-specific fecundity and temporal patterns in parturition and

discuss their effect on reproductive success in a population undergoing truncation of the upper end of its age distribution.

Materials and methods

We collected female black rockfish during the months of peak female reproductive development, November through March, for three successive years from 1995–96 through 1997–98. Female black rockfish were primarily obtained from recreational charter boat landings in Newport, Depoe Bay, and Charleston, Oregon, in addition to some fish from commercial landings from Port Orford, Oregon (Fig. 1). We also collected fish by rod and reel and spearfishing. When possible, the sex of all available black rockfish was determined, and females were staged as immature or mature. Immature females were measured (FL), and mature females were returned to the laboratory. On extremely busy days when numerous charter boats were fishing, all mature females were collected, but immature fish were not measured. In total we collected 1643 female black rockfish. Immediately upon return to the laboratory, we recorded fork length, total weight when possible (most samples from charter boats were carcasses only), liver weight, and ovary weight. Ovaries were assigned a maturity stage based on macroscopic appearance and preserved in 10% buffered formalin. We initially followed the gross maturity stage scheme of Nichol and Pikitch (1994) for darkblotched rockfish (*Sebastes crameri*) but ultimately abandoned their classification of maturity stages in favor of the simplified maturity stages reported by Gunderson et al. (1980) (Table 1). Sagittal otoliths were removed and stored dry for age determination. All aging was done by an expert age reader from the Oregon Department of Fish and Wildlife (ODFW), who used the break-and-burn technique (Beamish and Chilton, 1982). Ten percent of the otoliths were randomly selected for a second reading to ensure consistency in interpretation of annuli. It should be noted that black rockfish ages have not been validated. However, ages have been validated for yellowtail rockfish (*Sebastes flavidus*), a closely related species (Leaman and Nagtegaal, 1987), by using otoliths; moreover, the break-and-burn aging method is widely accepted as valid for aging rockfish (MacLellan, 1997), and ages thus derived are routinely used in rockfish stock assessments. Because our sample included all mature females that we encountered, we used these data to estimate the age distribution of mature females in each time period, and the age distribution of parturition during each time interval.

Histological preparations were made from the ovaries of 175 females collected monthly from March 1996 through March 1997 to track seasonal ovarian development. Females collected in March 1996 and November 1996 through March 1997 were from our regular sampling program, whereas fish collected from April through October 1996 were obtained from Newport recreational charter boat landings. Females were randomly selected from each maturity stage observed each month and from as wide a range of ages as available (Table 2). Ovaries were embedded in paraffin, sectioned at 4–5 μm, and stained with gill-3 haematoxylin and eosin y solution.

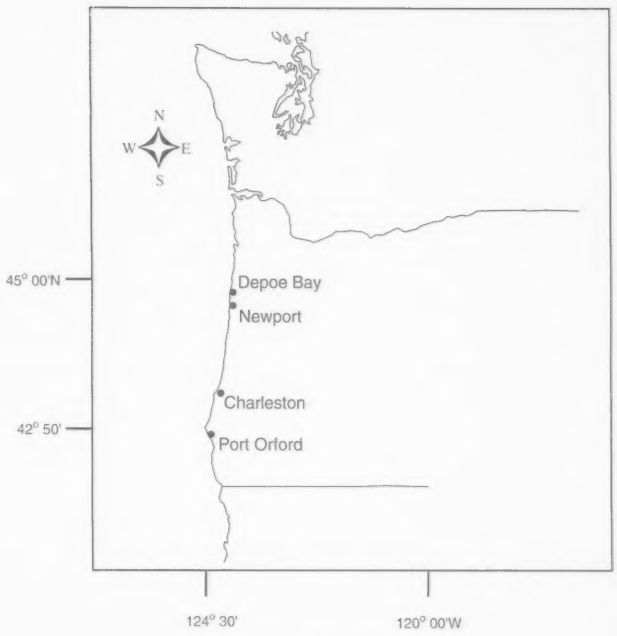

Figure 1

Map of the Oregon coast showing the study area where black rockfish (*Sebastes melanops*) were collected.

We determined stage-specific fecundity in black rockfish for females with unfertilized yolked oocytes (n=184) and fertilized eggs (n=85). Postfertilization ovaries were very fragile and tended to rupture easily and release embryos under the slightest pressure. Consequently, for estimating fecundity for these stages, we used only fish collected by ourselves so that we were certain that no eggs or larvae had been released during capture. To ensure that no eggs were lost after capture, these fish were immediately placed into plastic bags in order to retain any eggs that might be extruded before the ovary could be processed. Ovaries were processed following procedures modified from Lowerre-Barbieri and Barbieri (1993) to separate eggs and embryos from connective tissue. Briefly, fixed ovaries were manually manipulated and rinsed with water through a 1-mm square mesh sieve, which retained most of the connective tissue, into another sieve with 0.75-mm mesh. Ovary connective tissue was retained in the coarse sieve, and freed eggs were collected in the fine-mesh sieve. Freed eggs were patted dry, weighed (nearest 0.1 g), and three subsamples were collected, weighed (nearest 0.001 g), and placed in 10% buffered formalin.

Table 1

Macroscopic and histological descriptions of stages used to describe female black rockfish maturity.

Maturity stage	Macroscopic description	Histological description
1 Immature	Small and translucent ovary, pink during months without sexual activity and yellowish (except for very small fish) during months with reproductive activity.	Oocyte cytoplasm intensely basophilic. Densely packed oogonial nests and developing oocytes, with larger oocytes containing small clear vesicles.
2 Vitellogenesis	Ovary firm and yellow or occasionally cream in color. Large range of size, but all with visible opaque eggs.	Oogonia and developing oocytes still visible, but ovary dominated by large oocytes with numerous small red-staining yolk globules.
3 Fertilization	Eggs are golden and translucent. Ovary extremely large in relation to body cavity. Ovary wall thin and easily torn.	Fertilized eggs ovulated and found within the ovarian cavity. Eggs have a single pink-staining yolk mass and clear oil droplet.
4 Eyed larvae	Eyes of developing embryos visible, giving ovary an overall greyish color. Ovary fills a large portion of the body cavity.	Presence of developing larvae with black pigmented eyes. Yolk mass absorbed in late-stage larvae, but oil droplet usually present.
5 Spent	Ovary flaccid, purplish-red in color. Eyed larvae may still be visible.	Early-stage oocytes loosely associated. Extensive network of blood vessels. Possibility of encountering residual larvae.
6 Resting	Ovary again firm and pink in color. Black spots may be visible.	Similar appearance to immature fish. Ovary wall slightly thicker in early summer.

Table 2

Monthly ranges for age, length, and maturity stage of black rockfish collected off Oregon from March 1996 through March 1997 for histological analysis.

Month	Age (yr) range	FL (mm) range	Maturity stage range	*n*
March	7–25	375–510	1, 4–6	10
April	7–18	364–447	1, 5–6	12
May	7–13	340–465	1 and 6	15
June	5–13	349–432	1 and 6	15
July	5–13	360–475	1 and 6	14
August	5–11	357–493	1–2, 6	15
September	6–16	366–488	1 and 2	12
October	5–16	357–420	1 and 2	11
November	5–11	355–434	1 and 2	16
December	5–14	365–439	1 and 2	10
January	6–17	369–473	1–4	16
February	7–17	378–464	1–5	17
March	6–13	380–467	1, 5–6	12

The number of ova in the subsamples were counted and absolute fecundity was estimated by using the following algorithm:

$$AF = EW\left(\frac{\sum_{i=1}^{3}\frac{SSC_i}{SSW_i}}{3}\right),$$

where AF = absolute fecundity, or the total number of eggs per female;
EW = weight of rinsed eggs (or larvae);
SSC_i = subsample count i, where i=1 to 3; and
SSW_i = subsample weight i, where i=1 to 3.

Relative fecundity (RF), based on gonad-free somatic weight was estimated by

$$RF = \frac{AF}{TW - GW},$$

where AF = absolute fecundity, or the total number of eggs per female;
TW = total weight; and
GW = gonad weight.

For our analyses of fecundity, we used only fish in which the number of eggs or larvae estimated from the three subsamples had coefficients of variation less than or equal to 5%, and for prefertilization eggs we used only females with average egg diameters of at least 450 μ to ensure inclusion of all developing oocytes. Only one cohort of developing oocytes is present in the ovary of

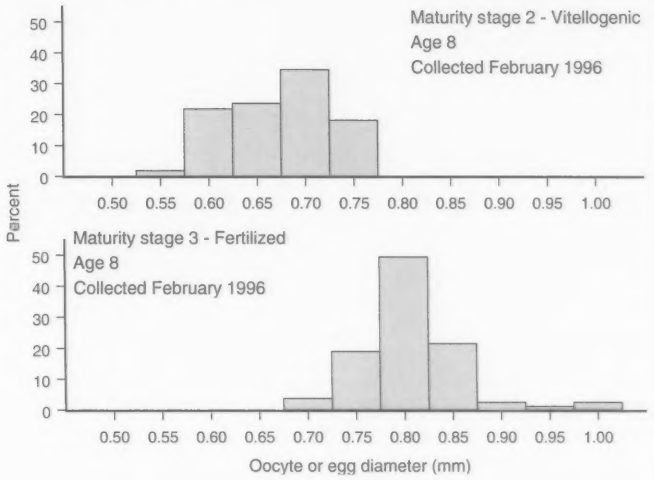

Figure 2

Prefertilization- and fertilized-egg–diameter frequency distributions showing a single mode of developing oocytes at both developmental stages.

black rockfish, either during development (stage 2) or after fertilization (stage 3) (Fig. 2). Analysis of covariance (ANCOVA) was used to test for annual effects in the relationship between prefertilization fecundity and age and a maturity-stage effect (prefertilization vs. fertilized-egg development stages) on both absolute and relative fecundity at age. We also used ANCOVA to test for a maturity stage effect in the relationship between absolute fecundity and fork length. All ANCOVA analyses were conducted by using multiple linear regression with the function lm in S-PLUS 2000 (MathSoft, Inc., Seattle, WA).

To predict the probability of a female black rockfish being mature based on its fork length, we fitted our maturity-at-length data to a logistic regression. During those months without reproductive activity, late spring through early fall, it was difficult to distinguish between immature and mature-resting ovaries. Consequently, only those females collected during the peak months of reproductive development and from sampling events where all fish, mature and immature, were collected were included in our analysis. Binary maturity observations (0=immature, 1=mature) and fork length were fitted to a logistic model by using the function glm, family = binomial of S-PLUS (S-PLUS 2000). The model used was

$$\pi(FL) = P(Y = 1 \mid FL) = \frac{e^{\beta 0 + \beta 1 FL}}{1 + e^{\beta 0 + \beta 1 FL}},$$

where $P(Y=1|FL)$ = probability of female black rockfish being mature at size FL; and

β_0 and β_1 = regression coefficients for the intercept and fork length, respectively.

For functional purposes, the response variable was interpreted as the percentage of female black rockfish mature at length. Assuming this relationship of fork length to maturity had not changed over time, we applied our logistic model to fork-length data from random sampling conducted by ODFW during the summers of 1992–2000 to calculate the percentage of female black rockfish caught by the recreational fishery off Newport that were mature in each year.

Fork length-at-age data for female black rockfish were fitted with the von Bertalanffy growth function (VBGF) by using the nonlinear function nls() in S-PLUS 2000. Age at 50% maturity was calculated by using our estimate of length at 50% maturity and a VBGF rearranged to the form

$$t_{50\%} = t_0 + \frac{1}{k}\left[\ln\frac{L_\infty}{L_\infty - l_{t50\%}}\right],$$

where $t_{50\%}$ = age at 50% maturity;
L_∞ = asymptotic length;
k = Brody growth parameter;
t_0 = age at zero length; and
$l_{t50\%}$ = length at 50% maturity.

Timing of parturition was estimated by microscopically determining embryo development stages for all females with fertilized eggs following Yamada and

Kusakari's (1991) stages of embryonic development for kurosoi (*Sebastes schlegeli*) modified to reflect the gestation period of 37 days for black rockfish (Boehlert and Yoklavich, 1984). Gestation period is likely to vary with water temperature. In determining gestation period, Boehlert and Yoklavich (1984) held black rockfish in the laboratory at 9–11°C. Mean water temperatures in our study area during the period of egg and larval development (December–April) were 10.9°, 10.1°, and 11.4°C in 1995–96, 1996–97, and 1997–98, respectively (http://co-ops.nos.noaa.gov/data). Even in the strong El Niño year of 1997–98, nearshore water temperature during the winter larval development period was only slightly outside this range. Therefore, we assumed a 37-day gestation period for all years of our study. Using the Boehlert and Yoklavich (1984) equation; ($stage\ duration = 0.0452 \times stage^{1.090}$) we solved for duration at each stage by adding 5 days to account for the time between hatching (stage 32) and parturition (also from Boehlert and Yoklavich, 1984). To calculate the time until parturition for each stage, we subtracted the previous stage durations from the total gestation period of 37. For example, at stage 1, parturition would occur in 37 days. At stage 2, parturition would take place in 37 days – stage-1 duration (~2 days) = 35 days.

For each year of our study, estimated parturition dates for all females in our sample were grouped into one-week time intervals and further subdivided into age categories: 6–8; 9–11; 12–14; and >15. These numbers were then multiplied by the appropriate value for age-class–specific fecundity based on fertilized eggs (Table 3) to estimate relative spawning output by week for each age class.

Table 3

Age group-specific absolute fecundity (based on fertilized eggs) and age distribution of mature females as a percentage of all mature females, used to estimate larval production. Calculated from data pooled from 1996 through 1998.

Age group (yr)	Absolute fecundity[1]	Percentage of all mature females represented by each age group
6–8	364,183.5	42.19
9–11	558,837.1	38.48
12–14	753,490.7	13.94
15 and older	948,144.3	5.39

[1] Absolute fecundity for each age group is the estimated fecundity (based on fertilized eggs) for ages 7, 10, 13, and 16, respectively.

Results

Ovarian development

Black rockfish off Oregon exhibited group-synchronous oocyte development; and females extruded only one brood of larvae per year (Fig. 2). Based on our observations of ovarian development from all three years of this study, parturition took place from mid-January through mid-March and peaked in February. Following parturition, unextruded larvae were quickly resorbed and the ovary lost much of its vascularization. From April through early August ovaries were in a resting state and contained oogonial nests and slightly larger oocytes with a basophilic cytoplasm and a maximum diameter of 50 μ. Also present at this time were developing oocytes ranging from 50 to 150 μ in diameter with small lipid vacuoles surrounding the nuclear membrane. Yolk deposition (vitellogenesis) began in late August and was observed through the third week of February. In the final stages of vitellogenesis, the largest oocytes were approximately 700 μ in diameter and had numerous oil vacuoles and yolk globules throughout the cytoplasm. The first female with fertilized eggs (stage 3) was observed during the second week of January, and stage-3 females were observed until the third week of February. Recently fertilized eggs were approximately 850 μ in diameter. The period of parturition as indicated by the occurrence of ovaries containing eyed larvae extended from the second week in January through the second week of March. Spent females were first collected during the last week of January and were most frequently collected in late February and early March.

Sexual maturity

Parameter values for the length-maturity logistic model were $\beta_0 = -26.73$ and $\beta_1 = 0.068$. The smallest mature female black rockfish we observed was 345 mm; all individuals were mature by 450 mm. Fifty percent of females were estimated to be mature at 394 mm fork length (Fig. 3). As reflected in our length-maturity logistic model, there was a decreasing trend in the percent maturity for female black rockfish in recreational landings from ODFW collections from 1992 through 2000 (Fig. 4). The von Bertalanffy parameter estimates for female black rockfish were $L_\infty = 442$ mm, $k = 0.33$, $t_0 = 0.75$ (Fig. 5). Using these estimates, along with the fork length at 50% maturity, we estimated the age at 50% maturity for female black rockfish to be 7.5 years. The median age of mature females decreased in each collection year from 10 years in 1996 to 9 in 1997 and to 7 years in 1998. In addition, we observed a significant decrease in the proportion of mature fish age 10 or older over the three years of our study (Pearson's $\chi^2 = 52.4$, df=2, $P<0.001$). The proportions decreased from 0.511 in 1996, to 0.318 in 1997, and 0.145 in 1998.

Fecundity

Absolute fecundity for prefertilization female black rockfish ranged from 482,528 oocytes for a 5-year-old female to 998,050 oocytes for a 19-year-old female. The results of ANCOVA (Table 4) over a common age

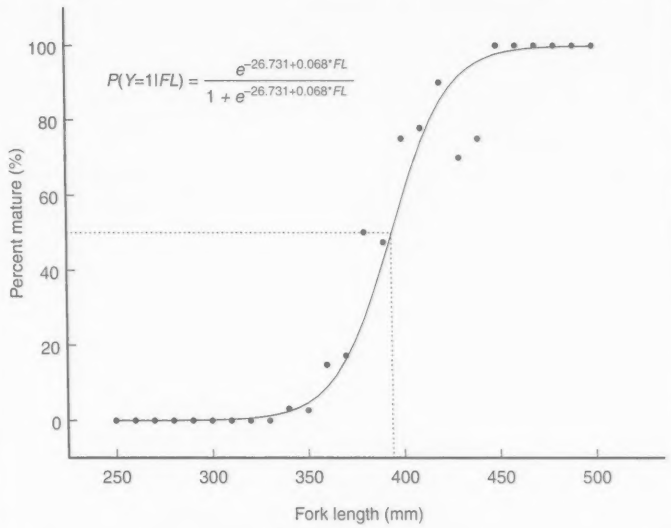

Figure 3

Logistic regression model for the estimated percentage of sexually mature female black rockfish as a function of fork length, with associated observed percent mature at 10-mm length intervals.

range showed no evidence of differences in slopes among the years 1996–98 (P=0.161). ANCOVA also showed no significant difference in elevations (P=0.632), indicating no annual effect and allowing one model to be fitted to the pooled data (Fig. 6). Absolute fecundity for females with fertilized eggs ranged from 299,302 embryos for a 6-year-old to 948,152 embryos for a 16-year-old. Because of the low number of females with developing embryos collected in 1996 and 1998, 19 and 4 females, respectively, and based on the results of prefertilization females, all data were pooled and fitted with one model (Fig. 7). Although we were able to pool the data for all years for fecundity-age regressions for both prefertilization females and fertilized females, there was evidence of interaction (i.e., unequal slopes) between stage-specific absolute fecundity and age (2-tailed t-test, P=0.020) requiring separate linear regressions to be fitted to the data (Fig. 8).

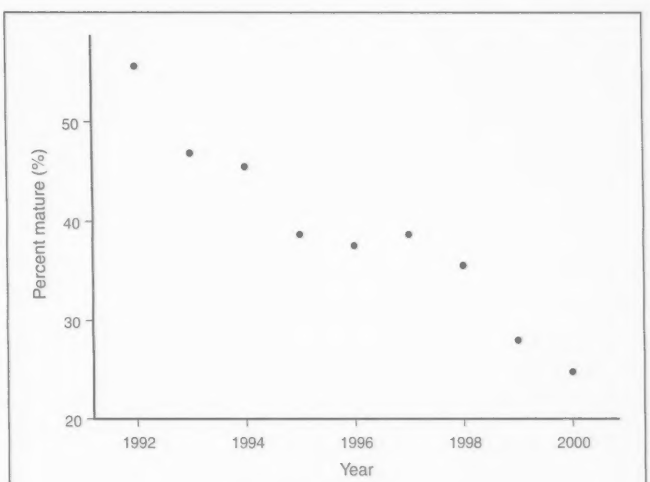

Figure 4

Estimated percent maturity for recreationally landed female black rockfish from Newport, Oregon, based on our logistic regression model of fork length on maturity. Data were from regular random summer port sampling conducted by the Oregon Department of Fish and Wildlife from 1992 through 2000.

Similar to the ANCOVA results for absolute fecundity, there were no differences in slopes or elevations for relative fecundity for prefertilization females for the years 1996–98 (Table 4). Again, based on the results of the ANCOVA for prefertilization females and due to the low number of fertilized females collected in 1996 and 1998, all relative fecundity data for females with fertilized eggs were pooled. Unlike the results for the relation between absolute fecundity and age there was no evidence of interaction (i.e., unequal slopes) between stage-specific relative fecundity and age (2-sided t-test

Table 4

Results of analyses of covariance testing for differences in slopes and elevations of annual absolute fecundity-age relation and annual relative fecundity-age relation. Response variables = AF and RF, treatment factors = year, and covariate = age.

Source of variation	df	Sum of squares	Mean square	*F*	*P*
Absolute fecundity (based on prefertilization oocytes)					
Equality of slopes	2	105,347	52,674	1.85	0.161
Error	160	4,564,320	28,527		
Equality of elevation	2	168,544	84,272	0.29	0.747
Error	162	3,459,048	288,254		
Relative fecundity (based on prefertilization oocytes)					
Equality of slopes	2	259.04	129.52	0.58	0.559
Error	160	35,452.22	221.58		
Equality of elevation	2	799.52	399.76	1.81	0.166
Error	162	35,711.26	220.44		

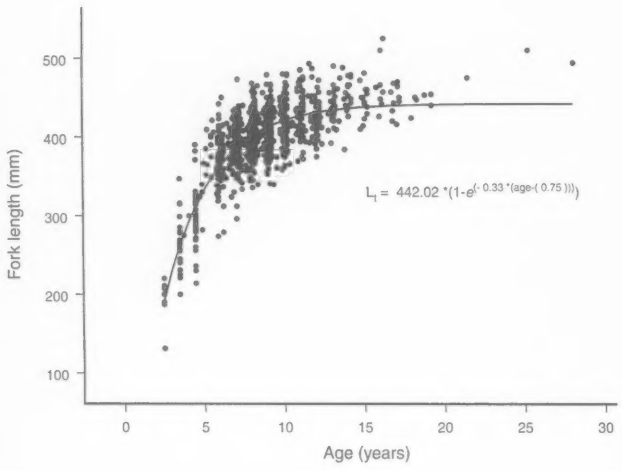

Figure 5

Fork length at age fitted to the von Bertalanffy growth model for female black rockfish.

P=0.096). There was, however, strong evidence (2-tailed t-test, P<0.001,) of a stage effect (i.e., unequal elevations) which necessitated that the data be fitted with a parallel-lines multiple linear regression model (Fig. 9).

Absolute fecundity for prefertilization female black rockfish ranged from 443,671 oocytes for a 381-mm-FL female to 1,135,457 oocytes for a 495-mm-FL female. For fertilized females, absolute fecundity ranged from 283,618 oocytes for a 381-mm-FL female to 1,073,356 oocytes for a 510-mm-FL female. The results of ANCOVA over a common size range showed no evidence of differences in slopes between maturity stages (2-sided t-test P=0.206). There was, however, strong evidence (2-tailed t-test, P<0.0001,) of a stage effect (i.e., unequal elevations) which necessitated that the data be fitted with a parallel-line multiple linear regression model (Fig. 10).

Temporal patterns in parturition

From 1996 through 1998 we estimated relative larval production for four age groups: 6–8; 9–11; 12–14; and 15 years and older (Fig. 11). In each year parturition took place from mid-January until mid-March, and older, larger fish extruded larvae earlier than younger fish. In 1996 and 1997, the 9–11 year-old fish dominated larval production, responsible for 60.1% and 49.6% of all larvae extruded, respectively (Table 5). In 1998 age 6–8 fish produced the largest percentage of larvae (65.3%). In all years, relative larval production was lowest for the oldest age group (15+), declining to near 0 by 1998.

Discussion

Ovarian development for black rockfish in Oregon was similar to the developmental cycles reported for other rockfish species (Moser, 1967; Bowers, 1992; Nichol and Pikitch, 1994) with the exception of seasonal timing and stage duration. Females underwent vitellogenesis for up to six months before fertilization, which occurred from December through February. In all three years, parturition off the Oregon coast occurred between mid-January and mid-March and peaked in February. Wyllie Echeverria (1987) observed similar timing for parturition of black rockfish off north-central California, with a peak in February but with parturition occurring through May.

All female black rockfish, except the smallest immature females, followed a seasonal cycle in which their ovaries developed an orange coloring during the months of reproductive activity—a pattern observed in olive rockfish (Love and Westphal, 1981). Similarly, Nichol and Pikitch (1994) observed darkblotched rockfish undergoing an "immature cycling" and even assigned these fish a maturity stage. After the reproductive season, the ovaries of immature black rockfish once again became pale pink in color. Because these fish were functionally immature and there was no way to project when they would become sexually mature, they were combined with those small, young females undergoing no seasonal ovarian development and were staged as immature.

Our estimate of fork length at 50% maturity for female black rockfish off Oregon was similar to the 400 mm estimate reported for north-central California females (Wyllie Echeverria, 1987), but lower than the estimate of 422 mm from Washington (Wallace and Tagart, 1994). Our estimated age at 50% maturity of 7.5 years was similar to the estimates of 7.9 and 7 years from Washington and north-central California, respectively. McClure (1982) reported that over 50% of examined female black rockfish collected off Depoe Bay, Oregon, were mature by age six. The difference between our estimate and McClure's was most likely due to using whole otoliths to age fish, which resulted in underestimates of age, and to assigning maturity stages only during summer months, which we have already described as problematic. Both absolute and relative fecundity increased with age for female black rockfish in Oregon waters, although there was a great deal of variation not accounted for by age. The low r^2 values for absolute fecundity regressions for pre- and postfertilization females (0.25 and 0.45 respectively) are due largely to the relatively poor correspondence between age and size (Fig. 5). Black rockfish, like many slow growing, long-lived fish grow slowly after sexual maturity. The rate of growth during their first few years can be quite variable depending on oceanographic conditions and food availability. As a result, young fish can be as large or larger than much older fish (Fig. 5). Length is a better predictor of fecundity than age as judged by the goodness-of-fit of the multiple linear regression model (Fig. 10; r^2=0.70).

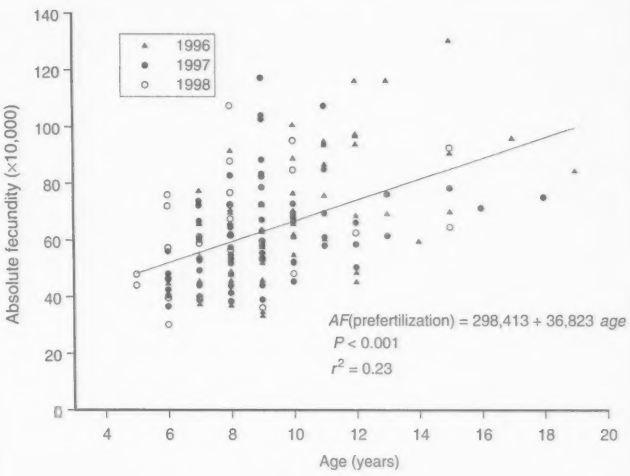

Figure 6

Scatter plot of black rockfish absolute fecundity (AF) (based on prefertilization eggs) on age by year (1996–98) with a fitted regression line from pooled data.

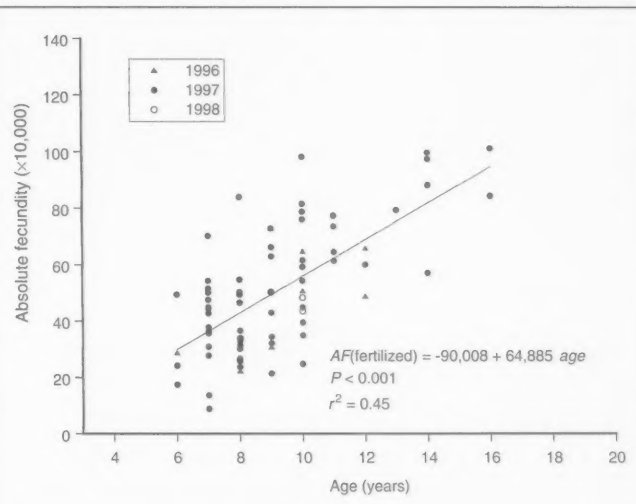

Figure 7

Scatter plot of black rockfish absolute fecundity (AF) (based on fertilized eggs) on age by year (1996–98) with a fitted regression line from pooled data.

An increase in absolute fecundity with age was observed in both prefertilization and postfertilization females, but they occurred at different rates. As illustrated in Figure 8, the absolute fecundity for a postfertilization 6-year-old black rockfish was only 58% of the estimated absolute fecundity for a prefertilization fish of the same age. By age 15 absolute fecundity

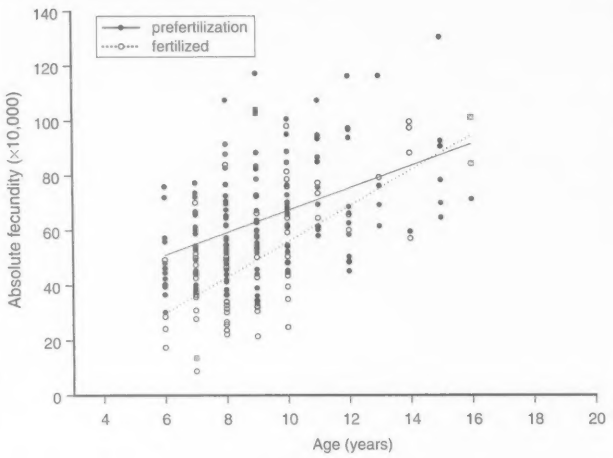

Figure 8

Separate-lines regressions fitted to absolute fecundity (based on prefertilization and fertilized eggs) on age for black rockfish in Oregon.

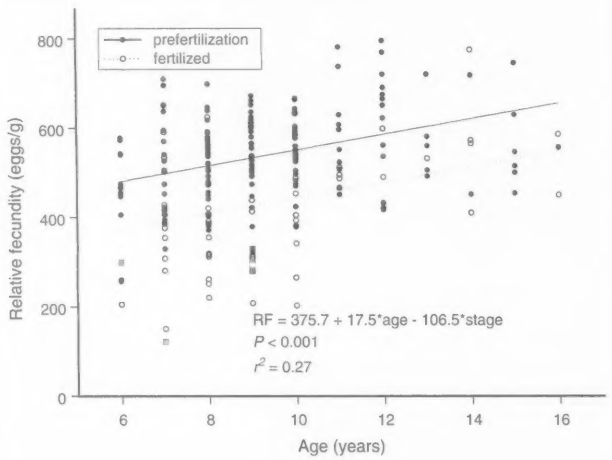

Figure 9

Parallel-lines model fitted to relative fecundity (based on prefertilization and fertilized eggs) on age for black rockfish in Oregon.

estimates for fertilized and prefertilization females were approximately equal. Yolked oocytes from older females were more successful in reaching the developing embryo stage. This may be attributed to higher rates of fertilization, greater viability of embryos, or a combination of both in older female black rockfish. Regardless of the mechanism there should have been signs of greater atresia in the ovaries of young fish, which we did not observe in our histological preparations. This may have been due to rapid resorption of unfertilized oocytes or an artifact of the fragile nature of fertilized ovaries, which made it difficult to obtain representative histological preparations. Nevertheless, these results suggest that fecundity in black rockfish is best described after fertilization, but care must be taken to minimize embryo loss. These results also suggest that current

estimates of reproductive potential, in which fecundity for prefertilization females is used, may overestimate actual larval production because an increasing proportion of the stock consists of young fish.

We observed a recurring trend of older, larger fish extruding larvae earlier in the reproductive season and larval output being increasingly dominated by younger and younger fish. Eldridge et al. (1991) reported that larger (and most likely older) yellowtail rockfish (*Sebastes flavidus*) spawned earlier in the season than smaller fish—a pattern also reported for darkblotched rockfish (*S. crameri*) by Nichol and Pikitch (1994). Reduced food availability has been suggested as a potential cause for delayed reproduction in *Sebastes* for smaller, younger individuals with high metabolic requirements for somatic growth (Larson, 1991). We feel that limiting the amount of energy that can be spent on reproductive development would cause lower fecundity or reduced yolk content, but not necessarily a delay in reproductive development that would result in suboptimal timing of parturition.

Stock assessments rarely consider changes in population age composition resulting from the removal of older age classes except to the extent that total egg and larval production is reduced. The decreasing representation of mature female black rockfish age 10 and older in the three years of our study indicates that age truncation is occurring in black rockfish in Oregon. This truncation not only removes biomass and potential larval production, but truncation of the upper end of the age distribution eliminates mature females with higher fecundity per individual, a greater success in carrying eggs through to the larval stage, and an age group that extends the overall parturition season. Further research is necessary to explore the controlling mechanisms of differential reproductive success with age and to determine how best to incorporate these findings into stock assessment models.

Acknowledgments

Many individuals contributed to the completion of this study. Tom Rippetoe provided many hours of expert assistance in all aspects of this research. Bob Mikus aged all of the adult black rockfish for this study. We thank him and the Oregon Department of Fisheries and Wildlife for all their support. We thank Dan Detman for his help and time in collecting black rockfish. We also thank Brock McLeod, Jason Castillo, David Stewart, and Michael Hogansen for all their help. We are especially grateful for all those unpaid volunteers who helped with fieldwork: Joe O'Malley, Mark Amend, Bill Pinnix, Wolfe Wagman, and Pat McDonald.

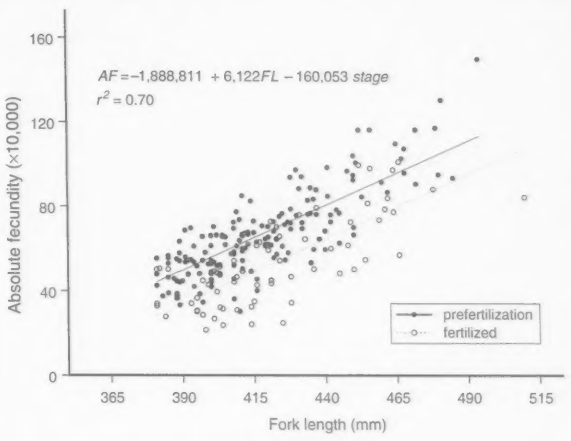

Figure 10

Parallel-lines model fitted to absolute fecundity (based on prefertilization and fertilized eggs) on age for black rockfish in Oregon.

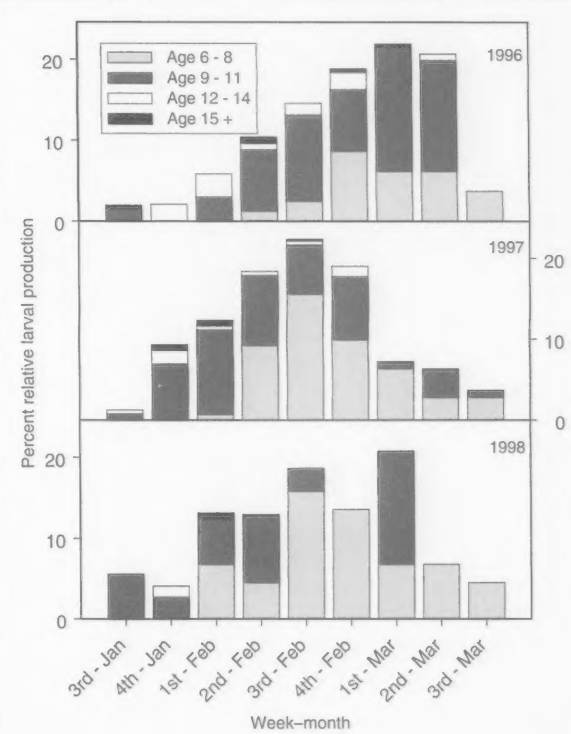

Figure 11

Percent relative larval production estimated from observations of larval development and age-group–specific absolute fecundity (based on fertilized eggs) for all mature females belonging to each age group of black rockfish collected in Oregon during 1996–98.

Table 5

Age group-specific relative larval production for 1996–98 for female black rockfish off Oregon.

	Percentage of relative larval production		
Age group	1996	1997	1998
6–8	26.4%	43.1%	65.3%
9–11	60.1%	49.6%	32.9%
12–14	11.3%	5.8%	1.8%
15 and older	2.2%	1.5%	0.0%

We thank the charter boat operators in Depoe Bay, Newport, and Charleston, Oregon, for their kindness, patience, and cooperation. Only through their assistance was this research possible. This research was partially supported by the NOAA Office of Sea Grant and Extramural Programs, U.S. Department of Commerce, under grant numbers NA36RG0451 (project no. R/OPF-46), and by appropriations made by the Oregon State legislature. Additional funding was provided through Hatfield Marine Science Center scholarships: the 1996 Barbara Schwantes Memorial Fellowship; the 1997 Mamie L. Markham Endowment Award; and the 1998 Bill Wick Marine Fisheries Award.

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Maori octopus (*Octopus maorum*) bycatch and southern rock lobster (*Jasus edwardsii*) mortality in the South Australian rock lobster fishery

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Abstract—Octopuses are commonly taken as bycatch in many trap fisheries for spiny lobsters (Decapoda: Palinuridae) and can cause significant levels of within-trap lobster mortality. This article describes spatiotemporal patterns for Maori octopus (*Octopus maorum*) catch rates and rock lobster (*Jasus edwardsii*) mortality rates and examines factors that are associated with within-trap lobster mortality in the South Australian rock lobster fishery (SARLF). Since 1983, between 38,000 and 119,000 octopuses per annum have been taken in SARLF traps. Catch rates have fluctuated between 2.2 and 6.2 octopus/100 trap-lifts each day. There is no evidence to suggest that catch rates have declined or that this level of bycatch is unsustainable. Over the last five years, approximately 240,000 lobsters per annum have been killed in traps, representing ~4% of the total catch. Field studies show that over 98% of within-trap lobster mortality is attributable to octopus predation. Lobster mortality rates are positively correlated with the catch rates of octopus. The highest octopus catch rates and lobster mortality rates are recorded during summer and in the more productive southern zone of the fishery. In the southern zone, within-trap lobster mortality rates have increased in recent years, apparently in response to the increase in the number of lobsters in traps and the resultant increase in the probability of octopus encountering traps containing one or more lobsters. Lobster mortality rates are also positively correlated with soak-times in the southern zone fishery and with lobster size. Minimizing trap soak-times is one method currently available for reducing lobster mortality rates. More significant reductions in the rates of within-trap lobster mortality may require a change in the design of lobster traps.

Manuscript submitted 28 April 2003 to Scientific Editor's Office.

Manuscript approved for publication 2 March 2004 by the Scientific Editor.

Fish. Bull. 102:430–440 (2004).

Fishing traps are used throughout the world to target a wide range of crustaceans, fishes, and cephalopods. Commercial trap fisheries, especially those for decapod crustaceans, are often the most valuable fisheries within a region (Phillips et al., 1994). Traps are generally considered to be an efficient and benign form of fishing because they appear to cause relatively minor damage to benthic habitats, can be designed to target particular species and size ranges, and produce live catches in good condition while minimizing bycatch (Miller, 1990).

There are 49 species of spiny lobsters (Decapoda: Palinuridae) worldwide, 33 of which support commercial trap fisheries. The largest of these are in Cuba, South Africa, Mexico, Australia, and New Zealand (Williams, 1988). The main trap fisheries in Australia are for western rock lobster (*Panulirus cygnus*) in Western Australia and southern rock lobster (*Jasus edwardsii*) along the southern coastline. Octopuses constitute a significant component of the bycatch in both fisheries (Joll[1]; Knight et al.[2]) .

In South Australia, *J. edwardsii* supports the State's most valuable commercial fishery. *Octopus maorum* is a significant bycatch species and is thought to be the major cause of lobster mortality in traps (Prescott et al.[3]).

Although the octopus bycatch of the South Australian rock lobster fishery (SARLF) is saleable, the commercial value of this product does not offset the value of the large number of lobsters that are killed in traps by octopus. Many fishermen are convinced that incidental mortality of octopus resulting from lobster fishing acts to control octopus numbers and that if these rates were reduced, octopus abundance and levels of within trap predation would increase.

Despite the prevalence of octopus bycatch in lobster fisheries, there have been only a few studies on the interaction between octopus and lob-

[1] Joll, L. 1977. The predation of trap-caught western rock lobster (*Panulirus Longipes cygnus*) by octopus. Department of Fisheries and Wildlife, Western Australia, Report 29, 58 p. [Available from Department of Fisheries, 168–170 St George's Terrace, Perth, Western Australia, 6000.]

[2] Knight, M. A., A. Tsolos, and A. M. Doonan. 2000. South Australian fisheries and aquaculture information and statistics report. Research Report Series 49, 69 p. [Available from SARDI Aquatic Science, 2 Hamra Avenue, West Beach, South Australia 5022.]

[3] Prescott, J., R. McGarvey, Y. Xiao, and D. Casement. 1999. Rock lobster. South Australian Fisheries Assessment Series 99/04, 35 p. [Available from SARDI Aquatic Science, 2 Hamra Avenue, West Beach, South Australia 5022.]

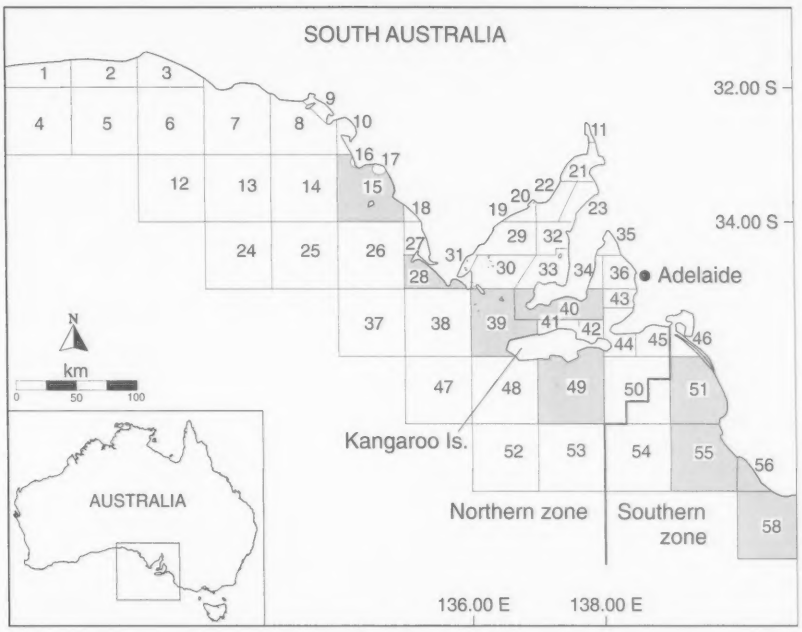

Figure 1

Map of the marine fishing areas (MFAs) of the South Australian rock lobster fishery. Shading shows the MFAs that were considered in this study and where most fishing effort is concentrated.

sters in traps (Joll[1]). Furthermore, there is a paucity of quantitative data on the impact of fishing on octopus populations, the proportion of lobster mortality that is attributable to octopus predation, or the long-term economic and ecological effects that octopus-induced mortality may have on lobster fisheries.

In this study, we examined the interaction between *O. maorum* and *J. edwardsii* in the South Australian rock lobster fishery (SARLF). The objectives were 1) to determine the number of lobsters and octopus caught and the number of lobsters killed in traps each year in the fishery; 2) to describe the interannual and seasonal patterns in lobster catch rate ($CPUE_L$), octopus catch rate ($CPUE_O$), and lobster mortality rate (M_L); 3) to examine some factors that may affect lobster mortality rates; 4) to estimate what proportion of the lobster mortality is attributable to octopus predation; and 5) to determine whether the rate of lobster mortality through octopus predation in traps is size dependent.

Materials and methods

South Australian rock lobster fishery

The SARLF is divided into a northern zone (NZ) and a southern zone (SZ), each of which is further divided into marine fishing areas (MFAs) for statistical purposes (Fig.1). There are 68 and 183 fishermen licensed to operate in the NZ and SZ respectively. The fishing season extends from November to May in the NZ and October to April in the SZ. A quota management system was introduced in the SZ in 1993, whereas the NZ is managed by gear restrictions and temporal closures.

Total annual catch and effort for the SARLF

Catch and effort data are recorded on a daily basis by all individual fishermen. Since 1983, a standardized logbook for recording catch and effort has been used across the fishery. Data provided by fishermen include MFA fished, average depth fished, number of trap-lifts, number and total weight of live lobsters, number of dead lobsters, and number and total weight of octopus. This information is stored in a South Australian rock lobster database that is managed by the South Australian Research and Development Institute, Aquatic Sciences.

Interannual and seasonal patterns in $CPUE_L$, $CPUE_O$, and M_L

Although commercial fishing for lobsters occurs along most of the South Australian coastline, the majority of effort is concentrated in only a few MFAs. In the NZ over the last 5 years about 72% of total trap-lifts were made in MFAs 15, 28, 39, 40, and 49. In the SZ over the same period 95% of trap-lifts were made in MFAs 51, 55, 56, and 58 (Fig. 1).

Data from the database were used to calculate catch rates of lobsters ($CPUE_L$), octopus ($CPUE_O$), and M_L on an annual and monthly basis for the nine major MFAs listed above. Catch rates from these MFAs for each fisherman were calculated according to the formula: *catch rate* = *catch number*/(*trap-lifts*/*day*). Annual and seasonal trends in $CPUE_L$, $CPUE_O$, and M_L were calculated for each zone and MFA.

Factors that affect within-trap lobster mortality

Potential factors that affect within-trap lobster mortality were analysed by using a general linear model (type-3 sums of squares) under the assumption that the number of dead lobsters follows a log-normal distribution.

The number of dead lobsters/trap-lift/day/license (with a ln+1 transformation) was used as the measure of lobster mortality. A model of the following structure was used to examine factors that affect the numbers of dead lobster:

$$\begin{aligned} \textit{Dead lobster} = {} & \textit{License} + \textit{MFA} + \textit{Month} + \textit{Year} \\ & + \textit{Effort} + \textit{Depth} + \textit{Octopus} + \textit{Lobster catch} \\ & + \textit{Soak-time} + (\textit{License}\times\textit{Year}) + (\textit{License}\times\textit{Month}) \\ & + (\textit{Year}\times\textit{Month}) + (\textit{Year}\times\textit{MFA}) + (\textit{Soak-time}\times\textit{Year}) \\ & + (\textit{Soak-time}\times\textit{Month}). \end{aligned}$$

In the model, *License* represents an individual fisherman, *MFA* is the marine fishing area, *Month* accounts for seasonal variation and *Year* accounts for interannual variation, *Effort* is the number of trap-lifts/license each day, *Depth* is the average depth fished by each License on a particular day, *Octopus* and *Lobster* are the respective daily catches/license, and *Soak-time* is the number of days that the traps remained in the water since the previous trap-lift.

The interaction terms *License*×*Year* and *License*×*Month* account for variations in the catch characteristics of the individual licenses over time that result from changes in fishing practises and efficiency associated with different boats, license holders, and skippers. The interaction terms *Year*×*Month* and *Year*×*MFA* account for variation in the population dynamics of octopus and lobster over time in different locations that could result in differential trends in lobster mortality. The interaction terms *Soak-time*×*Year* and *Soak-time*×*Month* reflects the change in general fishing strategies over time. In quota-managed fisheries the average soak-time will be affected by a number of factors, for example, that may include price, weather, and the fishermen's perceived ability to catch their quota.

The analysis was run separately for the SZ (n=493,629 traps) and NZ (n=155,628 traps) because the respective zones have different fishing seasons and management structures. The relationship between the number of dead lobsters and the factors depth, soak-time,and number of octopuses and lobsters were presented graphically by the equation:

$$\textit{Lobsters killed in traps} \propto \textit{factor}^{-\alpha},$$

where α = the parameter estimated by use of the model.

Source of lobster mortality and size-dependent mortality

A sampling program was conducted on three commercial vessels from the SZ during the 2001–02 fishing season. Five days were spent on each vessel. All lobsters caught were measured (carapace length, mm), and the sex (male or female), maturity (mature or immature), status (dead or alive), and cause of death (octopus or other) were recorded.

The method used to distinguish between lobsters killed by octopus or other means followed that of Joll.[1] This suitability of this approach was confirmed through examination of the carcasses of over one hundred lobsters killed by octopus in aquarium trials (Brock et al.[4]). Lobsters with shells that were partly or completely separated at the juncture between abdomen and cephalothorax but were otherwise undamaged were deemed to have been killed by octopuses, whereas lobsters with shells without this separation and with evidence of bite marks were deemed to have been eaten by other predators (fish or cuttlefish).

Anecdotal evidence from fishermen suggests that larger lobsters are more susceptible to predation than smaller ones. The effect of CL on the probability of mortality was examined separately for males and females by generalized linear modeling. The probability of mortality at a given size was modeled with a logistic equation of the form:

$$P(sex,\ CL) = 1/(1+e^{-(a+bCL)}),$$

where P(sex, CL) = the probability of a lobster of a given sex at carapace length CL being dead; and
a and b are parameters to be estimated.

Results

Estimation of total lobster catch, octopus bycatch, and lobster mortality

In 1999, there were 1.6 million trap-lifts in the SARLF, and 70% of this total effort was in the SZ (Fig. 2). The number of traps-lifts in the SZ declined from 2.2 million in 1983 to 1.2 million in 1999 (Fig. 2A). In contrast, fishing effort in the NZ remained relatively consistent with 406,000 trap-lifts in 1983 and 480, 000 trap-lifts in 1999 (Fig 2B).

The total annual lobster catch has generally increased in each fishing zone since 1983 (Fig. 2, A and B).

[4] Brock, D. J., T. M. Saunders, and T. M. Ward. In review. A two-chambered trap with potential for reducing within-trap predation by octopus on rock lobster. Can. J. Fish. Aquat. Sci., 19 p. [Available from SARDI Aquatic Science, 2 Hamra Avenue, West Beach, South Australia 5022.]

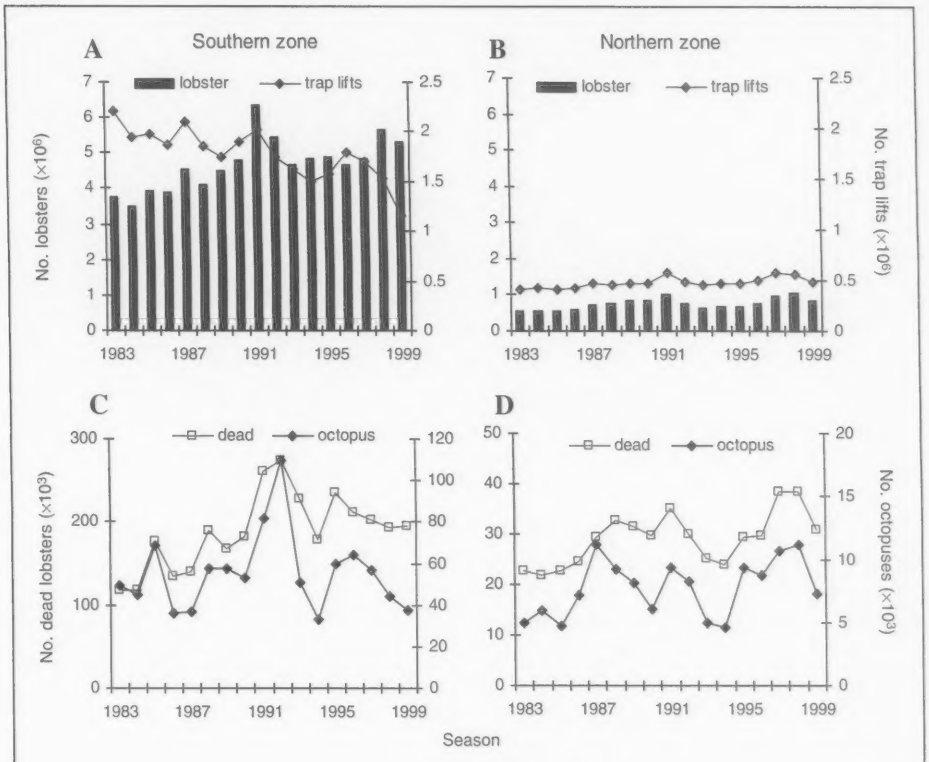

Figure 2

Annual total catch and effort for each zone of the South Australian rock lobster fishery for number of live lobsters caught (**A**), number of trap-lifts used (**B**), number of dead lobsters caught (**C**), and number of octopuses caught (**D**) (Note: change of scale in D).

In the SZ, the annual lobster catch rose from 3.8 million lobsters to a peak of 6.4 million lobsters in 1991 and was 5.4 million lobsters in 1999 (Fig. 2A). In the NZ, 560,000 lobsters were taken in 1983 compared to 850,000 in 1991 (Fig. 2B).

The total annual octopus catch varied among years in both zones, but between 70% and 90% of the total octopus catch were landed in the SZ (Fig. 2). The total number of octopus ranged from 36,000 in 1986 to 109,000 in 1992 (Fig. 2C) in the SZ, and from 4700 octopuses in 1985 to 11,200 in 1998 in the NZ (Fig. 2D).

In 1999, over 226,000 lobsters were killed in traps in the SARLF (Fig. 2). Since 1983, the mean proportion of dead lobsters out of the total catch has been approximately 4%. In the SZ, the number of lobsters killed in traps has generally increased from 118,000 in 1983 to 196,000 in 1999; a peak of 274,000 dead lobsters occurred in 1992 (Fig. 2C). In the NZ, there has also been a general increase in the number of lobster killed in traps each year; 24,000 dead lobsters were recorded in 1983, compared to 31,000 in 1999 and a peak of 39,000 dead lobsters recorded in 1998 (Fig. 2D).

Interannual and seasonal patterns in $CPUE_L$, $CPUE_O$, and M_L

Southern zone Mean annual $CPUE_L$ in the SZ increased from 175 to 466 lobsters/(100 trap-lifts/day) between 1983 and 1999, and the largest increase occurred between 1997 and 1999 (Fig. 3A). Mean annual $CPUE_O$ ranged from 1.8 to 6.2 octopus/(100 trap-lifts/day) in 1987 and 1992, respectively (Fig. 3C). Mean annual M_L rose from 5 to 17 dead lobster/(100 trap-lifts/day) between 1983 and 1999 (Fig. 3E). Peaks in both $CPUE_O$ and M_L occurred in 1985, 1992, and 1995.

Mean monthly $CPUE_L$ declined during the fishing season from 310 to 164 lobster/(100 trap-lifts/day) between October and April (Fig. 4A). In contrast, mean monthly $CPUE_O$ increased from 2.6 to 3.7 octopus/(100 trap-lifts/day) between October and December and declined to 1.8 octopus/(100 trap-lifts/day) in April (Fig. 4C). Similarly mean monthly M_L increased from 10.7 to 12.8 dead lobster/(100 trap-lifts/day) between October and November and declined to 6.7 dead lobster/(100 trap-lifts/day) in April (Fig. 4E).

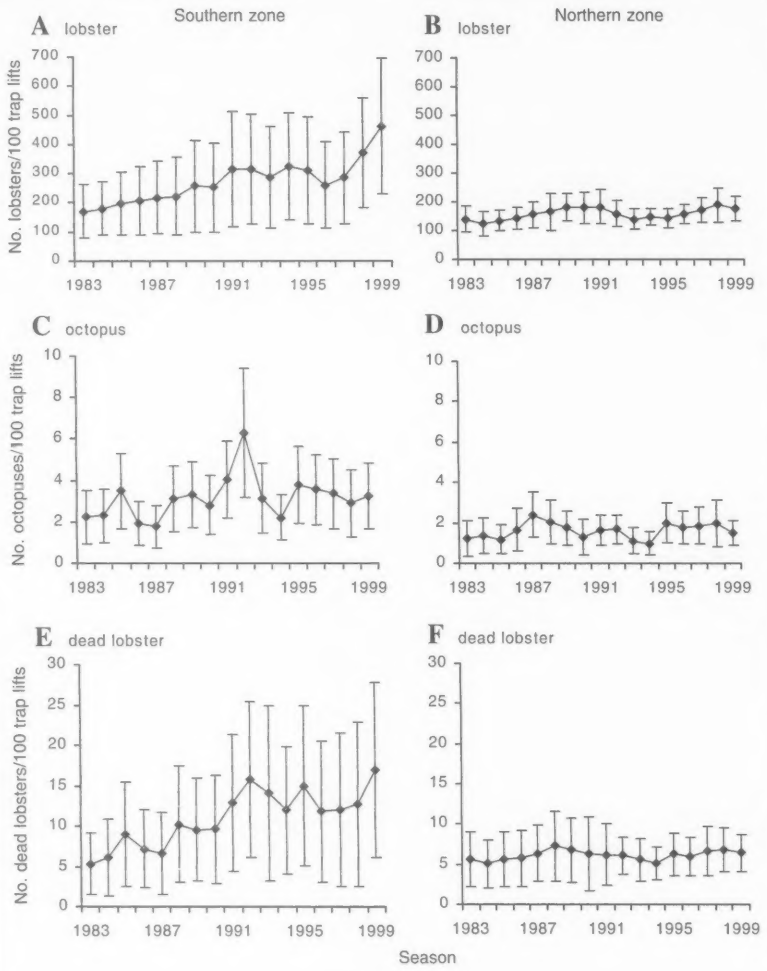

Figure 3

Annual catch rates in each fishing zone for lobsters ($CPUE_L$) (**A** and **B**), octopus ($CPUE_O$) (**C** and **D**), and dead lobsters (M_L) (**E** and **F**). Error bars are ±SD of mean.

Since 1983, mean annual $CPUE_L$ has increased in all MFAs, and has been consistently higher in MFAs 56 and 58 than in other areas (Fig. 5A). $CPUE_O$ has varied among years but has followed similar trends in different MFAs with consistent peaks in 1993 (Fig 5C). Prior to 1992, M_L was similar among MFAs but after 1992 was generally highest in MFAs 56 and 58 (Fig. 5E). M_L has increased over time in all MFAs.

Northern zone In the NZ, mean annual $CPUE_L$ rose from 135 to 179 lobsters/(100 trap-lifts/day) between 1983 and 1991, decreased to 138 lobsters/(100 trap-lifts/day) in 1993 and then rose again to 177 lobsters/(100 trap-lifts/day) in 1999 (Fig 3B). $CPUE_O$ ranged between 1.0 and 2.4 octopus/(100 trap-lifts/day) in 1987 and 1993 respectively (Fig. 3D). M_L ranged from 5.0 to 7.3 dead lobsters/(100 trap-lifts/day) in 1983 and 1988, respectively (Fig. 3F).

Mean monthly $CPUE_L$ declined from 196 to 88 lobster/100 trap-lifts/day between November and May (Fig. 4B). Mean monthly $CPUE_O$ was reasonably constant at between 1.43 and 1.7 octopus/100 trap-lifts/day for the first five months of the season before a decline to 1.0 octopus/100 trap-lifts/day in May (Fig. 4D). The mean monthly M_L declined from 7.5 to 3.4 dead lobster/100 trap-lifts/day between November and May (Fig. 4F).

Since 1983, mean annual $CPUE_L$ has been relatively low and stable in MFAs 15, 28, and 40 but has been

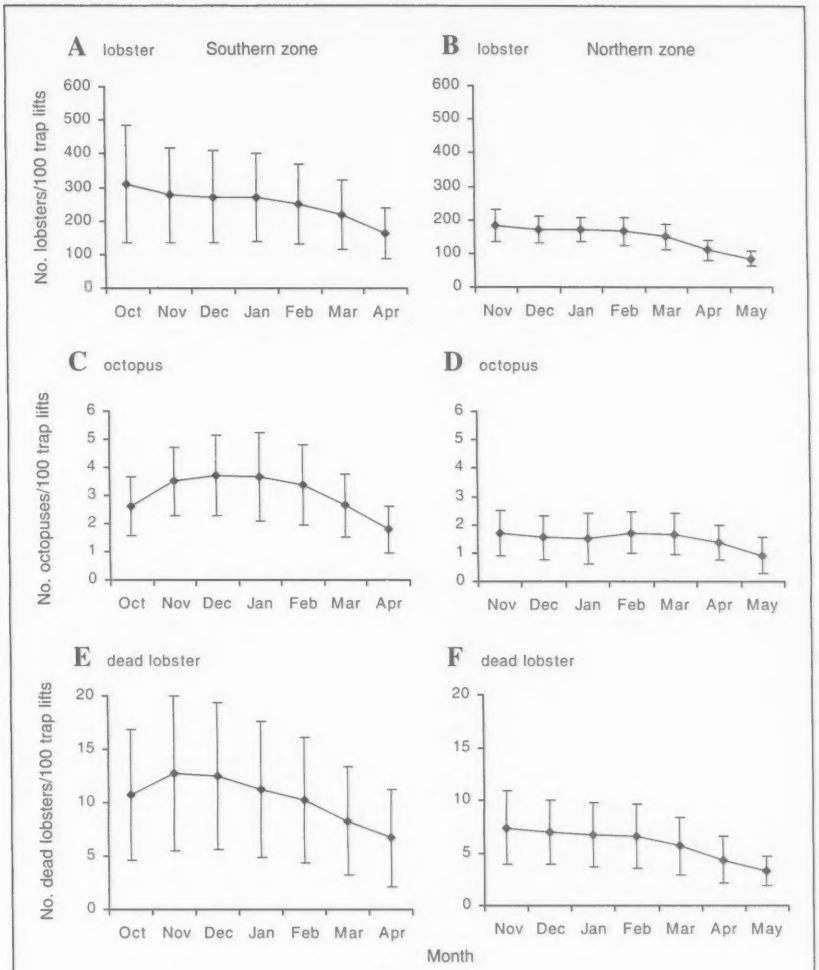

Figure 4

Mean monthly catch rates in each fishing zone for lobsters ($CPUE_L$) (**A** and **B**), octopus ($CPUE_O$) (**C** and **D**), and dead lobsters (M_L) (**E** and **F**). Error bars are ±SD of mean.

higher and more variable in MFAs 39 and 49 (Fig. 5B). There were large interannual fluctuations in $CPUE_O$ in each MFA, and these trends were similar among MFAs (Fig. 5D). M_L was highest in MFA 40, where a maximum of 12.5 dead lobsters/(100 traps lifts) was recorded 1998 and lowest in MFA 15 where the maximum was 5.2 dead lobsters/100 trap-lifts in 1997 (Fig. 5F). No clear long-term trends in M_L were apparent in any MFA.

Factors that affect within-trap lobster mortality

Based on the mean square values, the number of octopus had the greatest effect on lobster mortality in both zones (Table 1, A and B). The number of dead lobsters increased with both octopus and lobster catches and with soak-time and decreased as depth increased (Figs. 6 and 7). Based on the relative size of the mean square values, the factor with the greatest effect on the number of dead lobsters in the SZ was the number of octopus caught, followed by soak-time, number of lobsters caught, and depth. In the NZ, the number of octopus caught was also the most important factor, followed by the number of lobsters caught, depth, and soak-time.

Source of lobster mortality and size-dependent mortality

A total of 3627 lobsters from 635 trap-lifts were measured. In the sample there were 212 lobsters killed in traps of which 207 (98%) were killed by octopus and 5

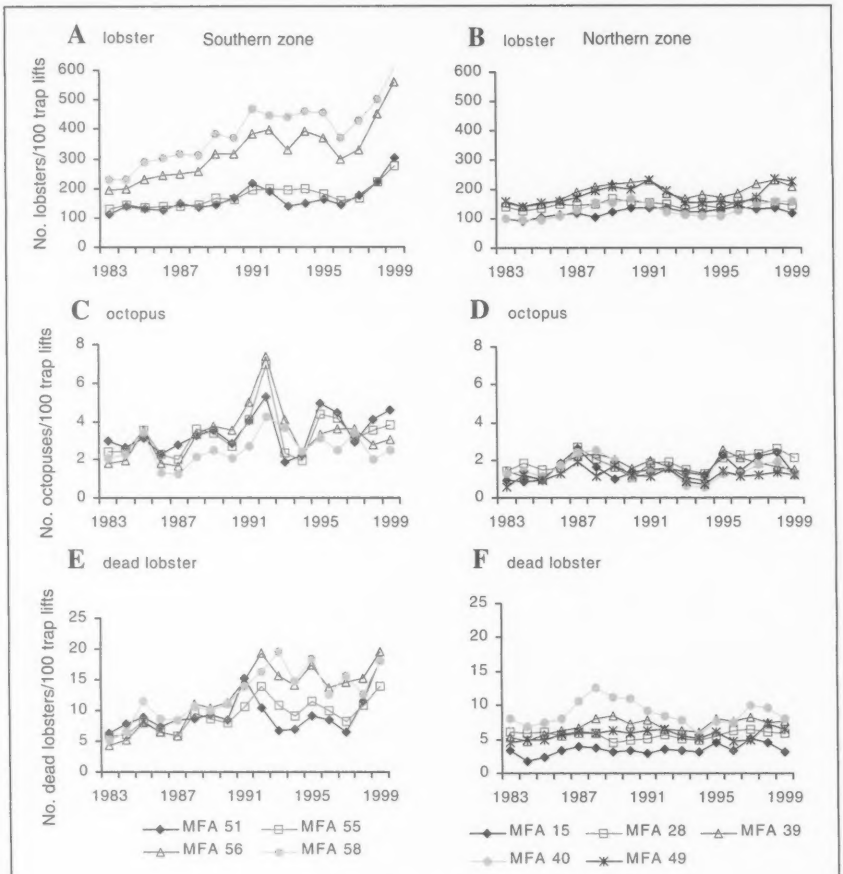

Figure 5

Annual catch rates of the major MFAs in each fishing zone for lobsters ($CPUE_L$) (**A** and **B**), octopus ($CPUE_O$) (**C** and **D**), and dead lobsters (M_L) (**E** and **F**).

by other predators. The mean CL of dead male lobsters was greater than live males (120 ±21.1(SD) vs. 110 ±18.3 (SD) mm, $P<0.001$). There was no significant difference in the mean size of live and dead female lobsters. For both sexes the probability of mortality increased with size according to the following relationships:

$$P\,(M_L, \text{males}) = 1/1+e^{-(-5.04+0.02CL)}$$
$$P\,(M_L, \text{females}) = 1/1+e^{-(-4.18+0.01CL)}.$$

Above 100 mm CL, the probability of mortality increased more sharply for male lobsters than for female lobsters (Fig. 8).

Discussion

Logbook data from the SARLF show that over the last five years approximately 240,000 lobsters have been killed in traps each year. Although there are numerous predators of trapped lobsters—such as seals, conger eels, and several species of finfish—the impacts of these taxa appear to be minor compared to the effects of predation by *O. maorum*. The field-sampling program conducted in the SZ in 2001–02 suggested that over 98% of within-trap mortality was attributable to *O. maorum*. Although the sampling program was spatially and temporally restricted, this finding, in conjunction with the strong correlations between annual, seasonal and spatial trends in the $CPUE_O$ and M_L, clearly demonstrates that *O. maorum* is the major predator of lobsters in SARLF traps.

The results of this study suggest that about 4% of the total annual catch of the SARLF is lost to predation by *O. maorum* in traps. Mortality rates attributable to octopuses in other Australian lobster fisheries range from 1% in the Western Australian fishery for *Panulirus cygnus*[1] (*O. tetricus*) to 5% in the Tasmanian fishery for

Table 1

Results of the general linear model of factors that affect lobster mortality (all data log transformed) for (**A**) southern zone (r^2=0.62), (**B**) northern zone (r^2=0.38).

A

Source	df	Squares	Mean square	*F*-value	*P*>*F*
Model	5319	314,536	59.13	147.41	<0.0001
Error	483,961	194,140	0.401		
Corrected total	489,280	508,677			

Source	df	Type-3 SS	Mean square	*F*-value	*P*>*F*
License	245	49158.1	200.6	500.2	<0.0001
MFA	3	9.6	3.2	8.0	<0.0001
Year	17	2494.6	146.7	365.8	<0.0001
MFA×Year	51	233.0	4.7	11.6	<0.0001
Month	6	2830.7	471.8	1176.1	<0.0001
Effort	1	229.6	229.6	572.4	<0.0001
Lobster catch	1	6728.7	6728.7	16773.4	<0.0001
Depth	1	1335.0	1335.0	3327.9	<0.0001
Octopus	1	35930.5	35930.5	89568.8	<0.0001
Soak-time	1	6842.1	6842.1	17056.1	<0.0001
Soak-time×Year	17	286.9	16.9	42.1	<0.0001
License×Year	3415	53019.8	15.5	38.7	<0.0001
License×Month	1460	5900.2	4.0	10.1	<0.0001
Year×Month	94	3760.4	40.0	99.7	<0.0001
Soak-time×Month	6	310.2	51.7	128.9	<0.0001

B

Source	df	Squares	Mean square	*F*-value	*P*>*F*
Model	2159	39,217	18.17	41.75	<0.0001
Error	148,731	64,713	0.435		
Corrected total	150,890	103,931			

Source	df	Type-3 SS	Mean square	*F*-value	*P*>*F*
License	95	3361.5	35.4	81.3	<0.0001
MFA	4	174.8	43.7	100.4	<0.0001
Year	17	241.2	14.2	32.6	<0.0001
MFA×Year	68	175.4	2.6	5.9	<0.0001
Month	7	317.3	45.3	104.2	<0.0001
Effort	1	27.1	27.1	62.3	<0.0001
Lobster catch	1	1299.7	1299.7	2987.2	<0.0001
Depth	1	391.3	391.3	899.3	<0.0001
Octopus	1	6305.1	6305.1	14491.0	<0.0001
Soak-time	1	210.8	210.8	484.4	<0.0001
Soak-time×Year	17	117.7	6.9	15.9	<0.0001
License×Year	1287	7275.6	5.7	13.0	<0.0001
License×Month	553	1170.7	2.1	4.9	<0.0001
Year×Month	95	275.9	2.9	6.7	<0.0001
Soak-time×Month	6	2.8	0.5	1.1	0.3743

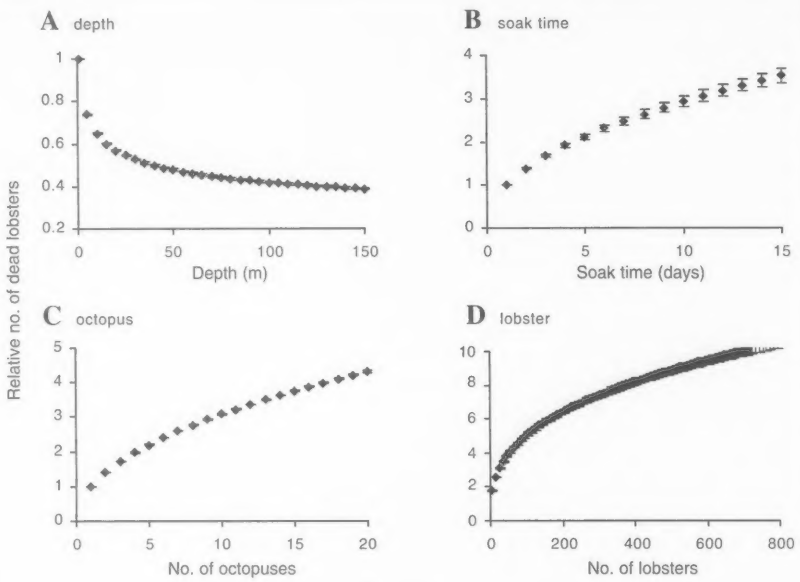

Figure 6

The relative number of dead lobster as a function of (**A**) depth, (**B**) soak-time, (**C**) number of octopus, and (**D**) no. of lobsters for the southern zone. Error bars are ±SD of mean.

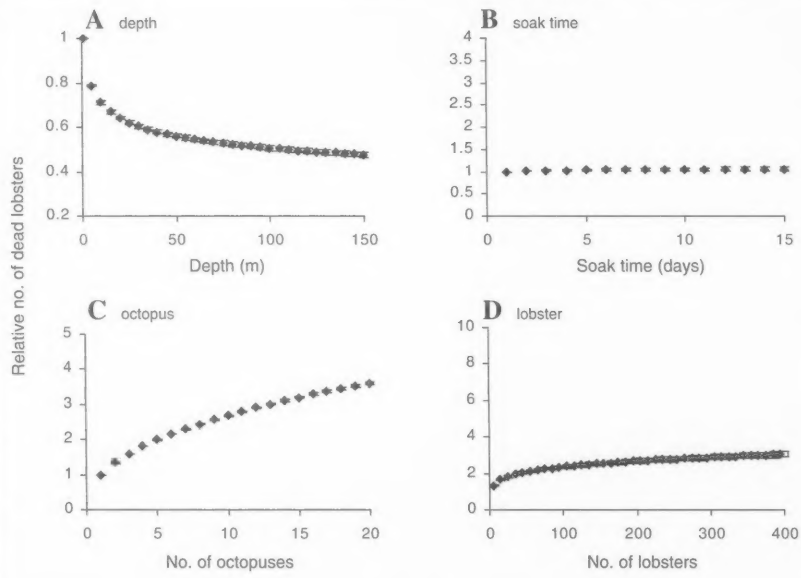

Figure 7

The relative number of dead lobster as a function of (**A**) depth, (**B**) soak-time, (**C**) number of octopus, and (**D**) number of lobsters for the northern zone. Error bars are ± SD of mean.

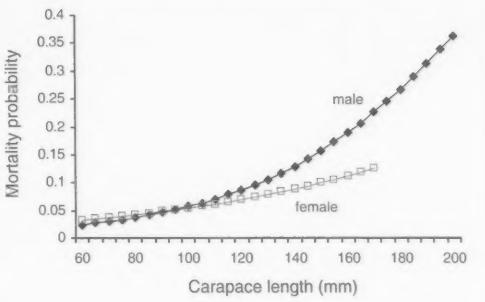

Figure 8

Size-dependent mortality of lobsters with respect to sex.

J. edwardsii (*O. maorum*), (Gardener[5]), and a localized study in the New Zealand fisheries for *J. edwardsii* (*O. maorum*) found the proportion of the lobster catch killed by octopus to be as high as 10% (Ritchie[6]). The estimates of lobster mortality from these other studies should be treated with caution however because the current study is the only one that documents within-trap lobster mortality from a fishery-wide data set.

The general linear modeling approach that we used to determine the factors associated with M_L has some limitations. For example, the logbook data for the SARLF, like the monitoring data for most other fisheries, are not completely independent, and interdependence among observations can bias estimates of parameters. Similarly, some of the factors in the model, notably $CPUE_L$ and $CPUE_O$ are partially correlated. In addition, the large number of observations and degrees of freedom tend to make most factors significant. We considered all of these issues when interpreting the results of the analyses and used the mean square (MS) values to rank the importance of factors.

In both zones, inter- and intra-annual fluctuations in M_L largely reflect the effects of $CPUE_O$ and $CPUE_L$. The broad trends in annual $CPUE_O$ have largely corresponded to those for M_L with peaks in both generally synchronous in both fishing zones. In the SZ, the general increase in M_L since 1983 appears to result from the increase in $CPUE_L$, which has more than doubled over this period. This assessment is supported by catch-rate data from individual MFAs. The two MFAs in the SZ that have had the greatest increases in $CPUE_L$ over the last 5 years (56 and 58) have also had the highest corresponding increase in M_L. Increases in $CPUE_L$ are likely to elevate M_L by both increasing the probability of octopus encountering traps containing lobsters and the number of lobsters in traps entered by octopus.

However, M_L is also positively correlated with soak-time, especially in the SZ. This finding is consistent with patterns observed in the New Zealand fishery for *J. edwardsii*[6] and reflects the increased opportunities for octopus predation when pots containing lobsters remain in the water for longer periods. In the SZ, fishermen return to port each day and choose to fish or not to fish each day according to factors such as weather and price; therefore, although a 24-h soak period is still most common, soak times can range from one to five days. In the NZ, fishermen remain at sea for extended periods and consequently soak times longer than 24 hours are rare.

There was considerable variation in the fishery data, especially in the southern zone. It is likely that much of this variation is related to the large geographical extent of the fishery as opposed to fishing practises. Across the fishery lobster growth rates and subsequent catch rates vary greatly (McGarvey et al., 1999a). For example, since 1991, the $CPUE_L$ in MFAs 56 and 58 have been twice those of MFAs 51 and 55 in the SZ. Although the variation in $CPUE_O$ between the zones has been similar, the higher variability in $CPUE_L$ in the SZ is reflected in the variation in M_L.

Data spanning 17 years and covering about 50,000 km^2 represent one of the few long-term and large-scale data sets on the distribution and abundance of an octopus species (Hernandez-Garcia et al., 1998; Quetglas et al., 1998). The paucity of octopus studies on these scales reflects the logistical constraints of fishery-independent surveys of octopus populations and the poor and inconsistent methods generally used to record fishery catch and effort data (Boyle and Boletsky, 1996). The few data that are available on the distribution patterns of octopus have been obtained mainly from small commercial fisheries and $CPUE_O$ has been included as a measure of relative abundance (Defeo and Castilla, 1998; Hernandez-Garcia et al., 1998). This approach has proven useful, but several potential biases must be considered when $CPUE_O$ data are being interpreted: these include 1) changes in fishing methods and efficiency over time; 2) the distribution pattern (e.g., random or aggregated) of the species under consideration; and 3) spatiotemporal fluctuations in catchability (Richards and Schnute, 1986; Rose and Kulka, 1999). There are several reasons why the data from the SARLF may provide a useful measure of the relative abundance of octopus over these spatial and temporal scales. Most importantly, the basic unit of effort in the fishery, the trap, has remained unchanged since 1983. Furthermore, although *O. maorum* is retained as bycatch and kills *J. edwardsii* in traps, it is neither targeted nor avoided by fishermen, and fishing effort is thus relatively independent of its distribution patterns because the economic effects of both the sale of octopus bycatch and the costs of lobster predation are relatively small compared to the primary

[5] Gardener, C. 2002. Personal commun. Tasmanian Aquaculture and Fisheries Institute, Private Bag 49, Hobart, Tasmania 7001.

[6] Ritchie, L. D. 1972. Octopus predation on trap-caught rock lobster—Hokianga area, N.Z. September–October 1972. New Zealand Marine Department. Fisheries Technical Report 81, 40 p. [Available from Ministry of Fisheries, 101–103 The Terrace, Wellington, New Zealand, 1020.]

economic driving force for the fishery, the lobster catch rates, and because the catch rates of octopus are difficult to predict. In addition, *O. maorum* is a solitary animal that tends to be dispersed randomly throughout areas of suitable habitat (Mather et al., 1985).

The higher total catches and catch rates of both lobster and octopus in the SZ, compared to the NZ, probably reflect the more extensive reef habitat and more intense nutrient-enrichment upwelling in this portion of the SARLF (Lewis, 1981). There have been large interannual fluctuations in $CPUE_O$ in both zones since 1983. Such fluctuations in population size are common among other cephalopods, especially squid, and may result from life history strategies that are characterized by rapid growth, short lifespan (<two years) and almost universal mortality after a single spawning event (Boyle and Boletsky, 1996). Despite these fluctuations, $CPUE_O$ has not declined noticeably in any MFA since 1983, which suggests that octopus mortality from fishing is consistent with little impact on octopus populations since the advent of fishing. This observation and the poor relationship between octopus catches and effort refute the belief of some SARLF fishermen that incidental fishing mortality acts to control octopus abundance.

This study, however, did confirm the view of fishermen that larger lobsters are killed more commonly by octopus than are smaller ones. This effect was most evident for male lobsters, which grow to larger sizes than females. There could be several reasons for the size-dependent mortality rates for rock lobsters. For example, octopus could actively select larger prey, or large lobsters could be captured more easily than small lobsters in traps by octopus. Because large lobsters can be worth more and produce more eggs than smaller lobsters, the increased mortality rates of large lobsters suggest that the total economic and ecological impacts of octopus predation in the SARLF are greater than indicated by the absolute number of lobsters killed.

Octopus predation of lobsters in traps is a significant problem in the SARLF. However, the economic effects vary between the zones. In the quota-managed SZ, additional lobsters are harvested to replace those killed in traps, which increases the time and costs of catch quotas, and imposes an impact on lobster abundance. In the input-controlled NZ, where there is no direct restriction on the quantity of lobsters taken, lobsters killed in traps represent both a direct economic loss and an impact on lobster abundance.

Like most fisheries for spiny lobsters, the SARLF is close to being fully exploited. Reducing rates of octopus predation provides one option available for increasing the value of the fishery. Some minor reductions in lobster mortality may be achieved by minimizing soak-times, especially in the SZ. More significant reductions in the rates of within-trap lobster mortality may be achieved by redesigning lobster traps (Brock et al.[4]).

Acknowledgments

This research was funded jointly by the Fisheries Research and Development Corporation, South Australian Rock Lobster Advisory Council, South Australian Research and Development Institute (Aquatic Sciences), and the University of Adelaide. The authors thank Thor Saunders for his support and assistance during the research. Jim Prescott provided advice for extracting data from the South Australian Rock Lobster Database. Yongshun Xiao provided statistical advice and assisted with the numerical modeling.

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Small-boat surveys for coastal dolphins: line-transect surveys for Hector's dolphins (*Cephalorhynchus hectori*)

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Abstract—Management of coastal species of small cetaceans is often impeded by a lack of robust estimates of their abundance. In the Austral summers of 1997–98, 1998–99, and 1999–2000 we conducted line-transect surveys of Hector's dolphin (*Cephalorhynchus hectori*) abundance off the north, east, and south coasts of the South Island of New Zealand. Survey methods were modified for the use of a 15-m sailing catamaran, which was equipped with a collapsible sighting platform giving observers an eye-height of 6 m. Eighty-six percent of 2061 km of survey effort was allocated to inshore waters (4 nautical miles [nmi] or 7.4 km from shore), and the remainder to offshore waters (4–10 nmi or 7.4–18.5 km from shore). Transects were placed at 45° to the shore and spaced apart by 1, 2, 4, or 8 nmi according to pre-existing data on dolphin density. Survey effort within strata was uniform. Detection functions for sheltered waters and open coasts were fitted separately for each survey. The effect of attraction of dolphins to the survey vessel and the fraction of dolphins missed on the trackline were assessed with simultaneous boat and helicopter surveys in January 1999. Hector's dolphin abundance in the coastal zone to 4 nmi offshore was calculated at 1880 individuals (CV=15.7%, log-normal 95% CI=1384–2554). These surveys are the first line-transect surveys for cetaceans in New Zealand's coastal waters.

Manuscript submitted 27 April 2003 to Scientific Editor's Office.

Manuscript approved for publication 3 March 2004 by the Scientific Editor.

Fish. Bull. 201:441–451 (2004).

Several international workshops on cetacean bycatch problems have stated that a key impediment to the conservation of coastal and riverine small cetaceans is the lack of quantitative data on abundance (e.g., IWC, 1994). An important reason for this lack of data is that line-transect surveys are often conducted from large (>50 m) vessels (e.g. Barlow, 1988) and hence are extremely expensive ($US 10,000/day). Such costs usually put high-quality surveys such as those conducted for harbor porpoise in the U.S. (e.g., Carretta et al., 2001) beyond the reach of less affluent nations. The need for abundance estimates is especially great for the coastal and riverine species found in Asia, Africa, Australasia, and South America (Table 1). Several of these species have apparently small populations and restricted distributions, and all suffer from being taken as bycatch in fishing gear, principally in gill nets (IWC, 1994). In addition, it is difficult or impossible for large vessels to work close to shore, in shallow waters, where some of these species are most common.

The work described in this contribution had two aims: 1) to adapt ship-based line-transect methods (e.g., Barlow, 1988) to a 15-m catamaran, and 2) to provide an updated estimate of the abundance of Hector's dolphin (*Cephalorhynchus hectori*). Hector's dolphin, a small delphinid found only in the inshore waters of New Zealand, is subject to bycatch in gill nets throughout its range (Dawson et al., 2001). At least in the Canterbury region, and off the North Island west coast, recent catch levels are clearly unsustainable (Dawson and Slooten, 1993; Martien et al., 1999; Slooten et al., 2000; Dawson et al., 2001). Studies of mt-DNA indicate that the very small North Island population is distinct and that there are at least three separate populations in South Island waters (Pichler et al., 1998; Pichler and Baker, 2000; see also Baker et al., 2002). At the time of the present study the only quantitative population estimate was from a strip-transect survey conducted in 1984–85 (Dawson and Slooten, 1988), in which the offshore distribution, as well as the

proportion of dolphins detected within the strip, was estimated. A current, more robust estimate is needed for management. This study describes line-transect boat surveys conducted to estimate Hector's dolphin abundance on the north, east, and south coasts of the South Island of New Zealand.

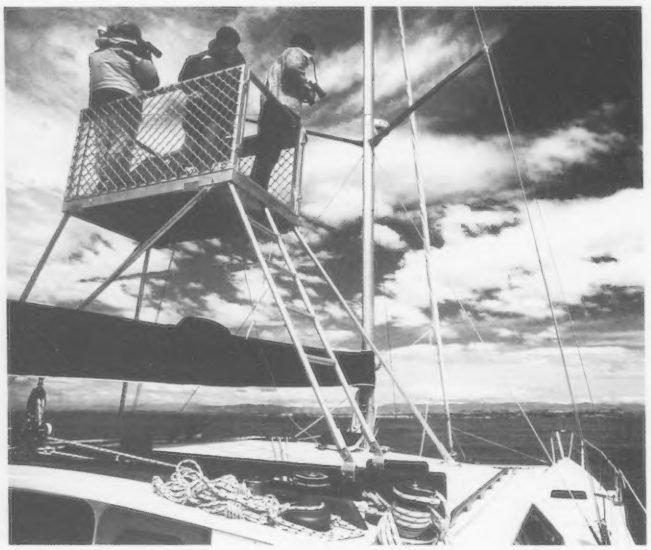

Figure 1
Photograph of the observer platform on the catamaran *Catalyst*.

Materials and methods

Vessel choice and field methods

Displacement catamarans are inherently suitable for inshore surveys because of their resistance to rolling and their ability to sustain reasonably high cruising speeds with modest power. We based our surveys from a 15.3-m sailing catamaran (RV *Catalyst*), which is powered by two 50-hp diesel engines, and cruises at 9–10 knots while using <10 liters of fuel per hour. We fitted a collapsible aluminum sighting platform (~6 m eye height; Fig. 1) to increase the resolution with which observers could measure the downward angles to sightings (see Lerczak and Hobbs, 1998, for details) and to allow the observers to see animals farther away. The surveys were conducted with a crew of six (five observers, one skipper).

Three people stood on the platform at any given time; one scanned the surface waters to the right of the platform, and the other scanned to the left, and a third person (the recorder) recorded sightings dictated by the observers. Sightings made by the recorder were not used in our analyses because his or her sighting effort was unavoidably uneven (the recorder could not make sightings while recording another sighting). The recorder did not point out sightings to observers. Observers and data recorder rotated

Table 1
Examples of coastal and riverine species of special conservation concern.

Common name	Scientific name	Habitat
Vaquita	*Phocoena sinus*	Northern Gulf of California
Chilean dolphin	*Cephalorhynchus eutropia*	Inshore coastal Chile
Hector's dolphin	*Cephalorhynchus hectori*	Inshore coastal New Zealand
Commerson's dolphin	*Cephalorhynchus commersoni*	Inshore coastal Chile, Argentina, Falkland Is, Kerguelen Is.
Heaviside's dolphin	*Cephalorhynchus heavisidii*	Inshore coastal South Africa and Namibia
Peale's dolphin	*Lagenorhynchus australis*	Coastal Chile, Argentina, Falkland Is.
Finless porpoise	*Neophocoena phocaenoides*	Coastal and riverine Asia and Indonesia
Indo-Pacific humpbacked dolphins	*Sousa chinensis*	Inshore tropical and estuarine habitats in western Pacific and Indo Pacific
Burmeister's porpoise	*Phocoena spinipinnis*	Coastal Chile, Argentina, Uruguay, Brazil
Franciscana	*Pontoporia blainvillei*	Coastal Brazil and Argentina
Indus river dolphin	*Platanista minor*	Indus River
Ganges river dolphin	*Platanista gangetica*	Ganges, Bramaputra, Karnphuli, Meghna rivers
Boto	*Inia geoffrensis*	Amazon River
Tucuxi	*Sotalia fluviatilus*	Coastal and estuarine Atlantic Central and South America

every 30 minutes to avoid fatigue. Although Hector's dolphins are easily identified from other species, and group size is typically small (usually 2–8; Dawson and Slooten, 1988), in order to maintain even sighting effort on both sides of the trackline, observers did not confer during a sighting. Sighting information was entered into a custom-written program on a Hewlett-Packard 200LX palmtop computer on the sighting platform. Data recorded included horizontal sighting angle, downward angle to sighting (in reticles), species, group size, orientation of the animals when first sighted, depth, Beaufort sea state, swell height, glare, GPS fix, date, and time. The program also recorded survey effort by storing a GPS fix every 60 seconds. Weather conditions were recorded at the start of field effort, and whenever they changed.

Observers used reticle- and compass-equipped Fujinon 7×50 (WPC-XL) binoculars to make sightings and to measure the downward angle from the land, or horizon, to the sighting. If the former, the corresponding distance to land was measured with RADAR (Furuno 1720 model), or, if within a few hundred meters of shore, with a Bushnell lightspeed laser rangefinder (tested accuracy ±1 m from 12 to 800 m). We calibrated the accuracy of the RADAR by comparison with transit fixes and laser rangefinder measurements. Sighting angles were recorded by using angle boards (see Buckland et al., 1993) in the first season, and thereafter with the compasses in the binoculars. There were no ferrous metals or significant electrical fields within 6 m of the sighting platform.

Navigation was facilitated by the use of a Cetrek 343 GPS chartplotter with digitized C-MAP charts onto which transect waypoints were plotted. Depths were measured with a JRC JFV-850 echosounder (at 200 kHz).

At the start of each survey, several days were spent training observers at Banks Peninsula, where sighting rates are high. Training continued until we gained about 100 sightings (data gathered in this period were not used in the analyses). An observer manual (available from authors) specified scanning behavior and recording methods. To ensure a wide shoulder on the histograms of perpendicular sighting distances, observers were instructed to concentrate their effort within 45° of the trackline and to spend less time searching out to 90°. Observers spent about 85% of the time scanning with binoculars. Regular scans with the naked eye minimized fatigue and reduced the chance of missing groups close to the boat. To promote consistency, observers were asked to re-read the manual at least once a week throughout the survey.

While the survey was underway, exploratory data analyses were undertaken to assess data quality. These analyses showed that in the early stages of the first survey, observers were rounding angles of sightings close to the trackline to zero. The use of the angle boards was modified to minimize this problem, and they were not used in subsequent surveys. The data from these early lines were discarded and the survey lines repeated.

Survey effort was restricted to sea conditions of Beaufort 3 or less and swell heights of ≤2 meters. Transect lines were run down-swell and down-sun to minimize pitching and effects of glare. Deviations of up to 10° from the intended course were made if needed to further reduce pitching or glare. The inshore end of each line was surveyed to just outside the surf zone on open coasts, or until a 2 m depth was reached, or to within 50 m of rocky shores. All surveys were conducted in passing mode to minimize the extent of vessel attraction.

Line-transect data were collected in three surveys in three consecutive summer seasons, each focussing on a particular coastal area (Fig 2; Motunau to Timaru, 5 January–21 February 1998; Timaru to Long Point, 9 December 1998–16 February 1999; Farewell Spit to Motunau, 17 December 1999–28 January 2000).

Survey design

In order to obtain a clear picture of density and to minimize variance in encounter rate, Buckland et al. (1993) recommend placing transects across known density gradients. Because short-distance, alongshore movements are well-known for Hector's dolphins (Slooten and Dawson, 1994; Bräger et al., 2002) and the dolphins' density declines sharply with distance offshore (Dawson and Slooten, 1988), transects were placed at 45° to the coast. On curved coastlines (within strata) we divided the coastline into blocks, drew an imaginary baseline along the coast, and placed lines at 45° to that baseline. The starting point of the first line along the baseline was decided randomly; thereafter lines were spaced at regular intervals according to the sampling intensity required in that stratum (Fig. 2). Within harbors we placed lines at 45° to an imaginary line down the center of the harbor (Fig. 3). The aim of this scheme was to ensure that, within a stratum, any one point had the same chance of being sampled as any other.

Survey effort was stratified according to existing data on distribution, obvious habitat differences, and areas of intrinsic management interest. In summer, very few Hector's dolphins are seen beyond four nmi from shore (Dawson and Slooten, 1988); therefore most sampling effort was placed in this inshore zone (i.e. 45° lines at 2-, 4-, or 8-nmi spacings, approximately proportional to density as determined from previous surveys). Within harbors, transect spacings were either one or two nautical miles. In the offshore zone (from 4 to 10 nmi) we expected very low densities, and therefore used sparse transect spacing (~30 nmi apart). It was not our intention to estimate density in this offshore zone. A subsequent aerial survey was found to be better suited for this purpose (Rayment et al.[1]).

Our goal was to estimate effective half strip width (ESW) separately for strata with different exposure to wind and swell. Hence, in each survey we aimed to gain sufficient sightings to estimate ESW separately for harbors or protected waters, and open coasts. To reach

[1] Rayment, W., E. Slooten, and S. M. Dawson. 2003. Unpubl. data. Department of Marine Science, Univ. Otago, P.O. Box 56, Dunedin, New Zealand.

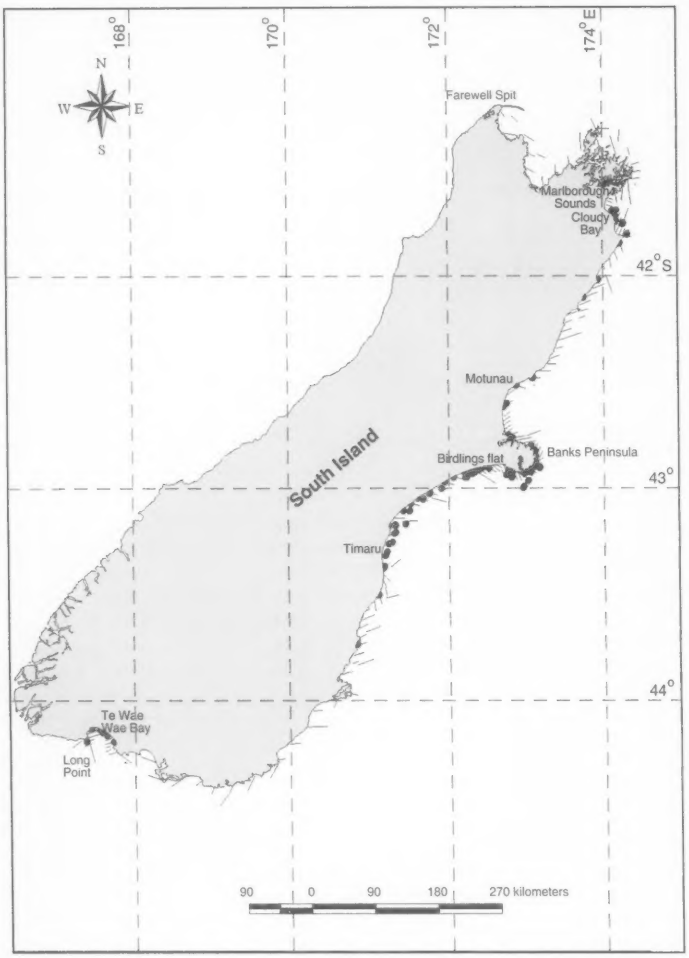

Figure 2

Map of New Zealand's South Island, showing transect lines and sightings of Hector's dophins (dots) 1997–2000.

Figure 3

Example of transect layout in harbors (1997–98 Akaroa Harbor transect lines and sightings, showing three replicates).

Buckland et al.'s (1993) target of 60–80 detections for robust ESW estimation, in the 1997–98 survey we conducted replicate surveys (with a new set of lines each time) in the harbors and bays stratum (e.g., Fig. 3). Low sighting rates in the area surveyed in 1999–2000 would have required unrealistic effort levels to reach this target; therefore we gained extra sightings from areas with the same exposure but higher sighting rates (e.g., data used to calculate ESW for the Marlborough Sounds were supplemented by data gathered in Akaroa Harbour by the same observers, in the same summer). Hence different sample sizes were available to estimate density and ESW (Table 2). Because observers changed between surveys, we did not pool sightings across years for estimating ESW. Strata areas were measured from nautical charts with a digital planimeter.

Data analysis

Within each stratum, Hector's dolphin abundance (N_S) was estimated as (Buckland et al., 1993):

$$\hat{N}_S = \frac{AnS}{2LESW}, \quad (1)$$

where A = size of the study area;
n = number of groups seen;
S = expected group size;
L = length of transect line surveyed, and
ESW = the effective half strip width.

Because there was no significant relationship between group size and detection distance, expected group size was estimated as a simple mean group size.

Table 2

Survey effort by stratum. Number of sightings is the total number made before truncation and quality auditing (see "Vessel choice and field methods").

Survey zone	Stratum	Survey effort (km)	No. of sightings	Sightings per km
Motunau to Timaru (1997–98)	Banks Peninsula harbors and bays	223	89	0.399
	Banks Peninsula Marine Mammal Sanctuary (BPMMS) (excluding open coasts)	265	66	0.249
	<4 nmi offshore, to the north and south of BPMMS	174	21	0.121
	Offshore (4–10 nmi)	89	4	0.045
Timaru to Long Point (1998–99)	Timaru–Long Point (excluding Te Waewae Bay)	336	13	0.04
	Te Waewae Bay	101	14	0.14
	Offshore (4–10 nmi)	106	0	0
Motunau to Farewell Spit (1999–2000)	Farewell Spit–Stephens Island	120	0	0
	Marlborough Sounds (including Queen Charlotte Sound)	205	3	0.015
	Cape Koamaru–Port Underwood	68	0	0
	Cloudy Bay and Clifford Bay	89	13	0.146
	Cape Campbell–Motunau	192	5	0.026
	Offshore (4–10 nmi)	93	2	0.022

Using the program Distance 3.5 (Research Unit for Wildlife Population Assessment, University of St. Andrews, UK), we fitted detection functions to perpendicular distance data to estimate ESW (note that this value is derived directly from $f(0)$). Akaike's information criterion (AIC) was used to select among models fitted to the data. Models and adjustments were the following: hazard/cosine, hazard/polynomial, half-normal/hermite, half-normal/cosine, uniform/cosine (Buckland et al., 1993). Following Buckland et al. (1993), perpendicular sighting distances were truncated to eliminate the farthest 5% of sightings and binned manually for $f(0)$ estimation.

The coefficient of variation (CV) for the abundance estimate was calculated from the coefficients of variation of each variable element in Equation 1 above (Buckland et al., 1993):

$$CV(\hat{N}_S) = \sqrt{CV^2(n) + CV^2(S) + CV^2(ESW)}. \quad (2)$$

The *CV(n)* was estimated empirically as recommended by Buckland et al. (1993):

$$CV(n) = \frac{\sqrt{\mathrm{var}(n)}}{n^2}, \quad (3)$$

where $$\mathrm{var}(n) = L\sum l_i(n_i / l_i - n / L)^2 / (k-1), \quad (4)$$

l_i = the length of transect line i;
n_i = the number of sightings on transect i; and
k = number of transect lines.

CV(S) was estimated from the standard error of the mean group size. *CV(ESW)* was estimated with the bootstrapping option in Distance 3.5 software. This process incorporates uncertainty in model fitting and model selection (Buckland et al., 1993).

Measuring the effect of attraction

Conventional line-transect estimates can be biased as a result of responsive movement of the target species and animals on or near the trackline being missed by observers (Buckland et al., 1993). Buckland and Turnock (1992) presented a method using co-ordinated boat and helicopter surveys to quantify and adjust for the combined effects of responsive movements of dolphins to the boat and to eliminate the bias from observers failing to see animals on or near the trackline. Their approach is better suited to the restricted space available on small boats than to a dual-platform approach (Palka and Hammond, 2001). Additionally, sightings can be made much farther ahead (reducing the possibility that the animals have already responded), and the two sighting teams are totally isolated from each other. For these reasons we adapted Buckland and Turnock's (1992) approach in our trials of 1998–99.

Simultaneous boat-and-helicopter surveys were carried out to the south of Banks Peninsula, predominantly between Birdlings Flat and the mouth of the Rakaia River. This area was chosen because it displayed representative and varying densities.

A Robinson R22 helicopter with pilot and one observer (ES) followed a zig-zag flight path approximately 1.5 km in front of the boat, traveling out to 1000 m on either side of the vessel's trackline at a height of 500 ft (152 m) (Fig. 4). To aid the process of tracking sightings from the air, sighting positions were marked with Rhodamine dye bombs.[2] The position of the helicopter in relation

[2] Dye bombs consisted of a tablespoon of Rhodamine dye in a paper cup 2/3 filled with sand. An additional (empty) paper cup was taped upside down on top of the first cup with paper-based masking tape. On impact the two cups broke apart, releasing the sand+dye mix into the water.

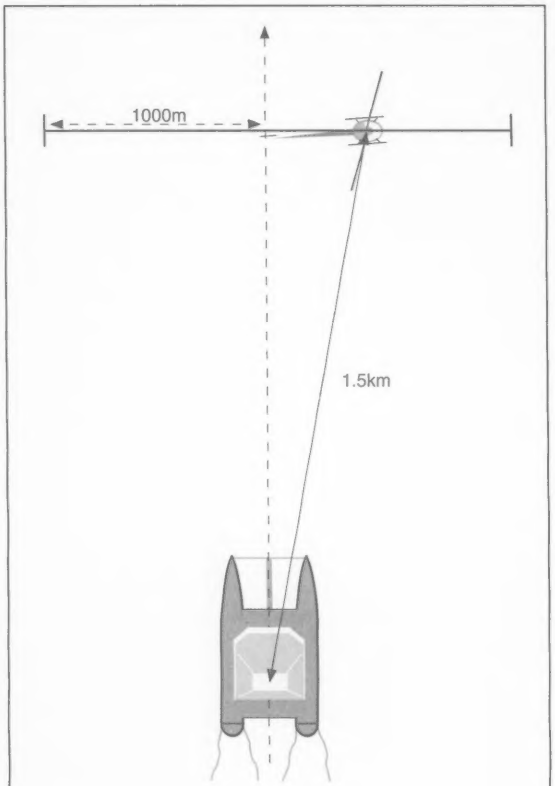

Figure 4

Schematic diagram of simultaneous helicopter-and-boat surveys for Hector's dolphins south of Bank Peninsula, South Island, New Zealand.

to the boat was determined with the boat's RADAR. The absolute position of the boat was determined to an accuracy of 2–5 m by differential GPS (Trimble GeoExplorer; postprocessed). Distances to land were obtained at the time of sighting with RADAR or during analysis by using GIS coastline data and the computer program "SDR Map" (Trimble Navigation, Christchurch, New Zealand).

Boat observers followed our standard sighting procedures (see above). On most occasions the helicopter was outside the field of view of the observers' binoculars because the observers were scanning the water surface, and the helicopter was well above what the observers could see through the binoculars. When it was within their view, observers made a conscious effort to remain unbiased by the movements of the helicopter. On making a sighting, the helicopter observer informed an independent observer located in the cabin (observers on the platform could not hear communications from the helicopter observer and vice versa). The helicopter then hovered briefly above the sighting while a range and bearing in relation to the boat was taken by RADAR. The helicopter then ceased hovering but tracked the group of dolphins either until the boat observers had sighted the group, or the group had passed abeam of the boat. A second range and bearing were then taken. Sightings lost by the helicopter observer during tracking were discarded in our analyses. The independent observer, in liaison with the helicopter observer and boat observers, determined whether the sighting was a duplicate (i.e., made by both helicopter and boat observers) by using information on location and group size. These decisions were checked again in analysis by inspection of plotted locations of sightings made from either platform or both platforms.

Following the approach of Buckland and Turnock (1992), let

$g_s(y)$ = the probability that a group detected from the helicopter at perpendicular distance y from the trackline of the ship is subsequently detected from the ship;

$$f_S(y) = g_S(y)/\mu, \text{ with } \mu = \int_0 g(y)dy$$

(area under helicopter detection function),

w = truncation distance for perpendicular distances y;

n_h = number of helicopter detections;

n_s = number of ship detections;

n_{hs} = number of detections made from both platforms (duplicate detections);

$f_h(y)$ = probability density function fitted to helicopter detection distances;

$f_{hs}(y)$ = probability density function fitted to duplicate detection distances as recorded from the helicopter;

$f(x)$ = probability density function fitted to perpendicular distances recorded from the ship;

L = length of transect line.

A conventional estimate of density of groups, assuming no responsive movement and $g(0) = 1$ (all animals on the trackline seen with certainty) is calculated as

$$\hat{D}_s = \frac{n_s \hat{f}(0)}{2L}. \quad (5)$$

A corrected estimate, allowing for responsive movement and including an estimate of $g(0)$ is given by

$$\hat{D}_U = \frac{n_s \hat{f}_s(0)}{2L\hat{g}_s(0)}, \quad (6)$$

where $$\hat{f}_s(0) = \frac{\hat{g}_s(0)}{\int_0^w \hat{g}_s(y)dy}, \quad (7)$$

$$\hat{g}_s(y) = \frac{n_{hs}\hat{f}_{hs}(y)}{n_h \hat{f}_h(y)}. \quad (8)$$

A correction factor for abundance estimates of Hector's dolphin groups can be estimated by

$$\hat{c} = \hat{D}_U / \hat{D}_S. \quad (9)$$

Using Distance 3.5, we fitted a half-normal model with cosine adjustments to estimate $f(0)$. The half-normal model was fitted to helicopter data to estimate $f_h(0)$ and the uniform model with cosine adjustments was used to estimate $f_{hs}(0)$. All were selected by using AIC. Potential model choices were the following: hazard/cosine, hazard/polynomial, half-normal/cosine, half-normal/hermite and uniform/cosine (Buckland et al., 1993). Truncation distance was 640 m for boat sightings, and 1000 m for helicopter and duplicate sightings. To ensure that only high-quality data were used to estimate effective half search widths, sightings for which range (radial distance) was estimated by eye and those made during Beaufort sea state >2 were removed before $f(0)$ estimation.

The error for the correction factor ($\hat{c}$) was estimated by bootstrapping on transect lines and applying the estimation procedure to each of 199 bootstrap data sets. The standard deviation of the bootstrap estimates was used as the standard error of $\hat{c}$.

Ideally, the correction factor would be estimated separately for each survey from separate sets of boat-and-helicopter trials conducted in areas of representative density. Financial and logistical constraints prevented this; therefore the correction factor estimated in 1998–99 was applied to each of the line-transect surveys reported in the present study. We note that this is not uncommon (e.g., Carretta et al., 2001).

Unbiased abundance estimates were calculated by

$$\hat{N}_U = \hat{c}\hat{N}_S. \quad (10)$$

The CVs of the corrected abundance estimates ($\hat{N}_U$) were calculated with the following equation (Turnock et al., 1995):

$$CV(\hat{N}_U) = \sqrt{CV^2(\hat{c}) + CV^2(\hat{N}_S)}, \quad (11)$$

$$\text{where } CV(\hat{c}) = \frac{SE(\hat{c})}{\hat{c}}. \quad (12)$$

Upper ($\hat{N}_{UC}$) and lower ($\hat{N}_{LC}$) 95% confidence intervals for $\hat{N}_U$ were calculated by using the Satterthwaite degrees of freedom procedure outlined in Buckland et al. (1993). This procedure assumes a log-normal distribution of N_C, using

$$\hat{N}_{LC} = \hat{N}_U / C, \text{ and}$$
$$\hat{N}_{UC} = \hat{N}_U C, \quad (13)$$

$$\text{where } C = \exp\left\{t_{df}(0.025)\sqrt{\log_e\left(1+\left[CV(\hat{N}_U)\right]^2\right)}\right\}. \quad (14)$$

The Satterthwaite degrees of freedom (df) for corrected abundance estimate confidence intervals were calculated by

Table 3

Summary of variables required for correction factor (boat-and-helicopter trials)

Parameter	Estimate
Length of transect, L (km)	308
Truncation distance, w (km)	1.0
Number of helicopter detections, n_h	58
Number of ship detections, n_s	126
Number of duplicate detections, n_{hs}	33
ESW of helicopter (km)	0.532
ESW for duplicates (km)	0.342
Apparent ESW of boat (km)	0.268
Apparent density estimate (groups/km^2)	0.7631
Corrected density estimate (groups/km^2)	0.3839
Boat detection probability "near" trackline	0.8861
Correction factor (c)	0.5032
Standard error, SE(c)	0.0912

$$df = \frac{CV^4(\hat{N}_U)}{\frac{CV^4(\hat{c})}{B-1} + \frac{CV^4(\hat{N}_S)}{df_S}}, \quad (15)$$

where B is the number of bootstrap samples, and df_S is the Satterthwaite degrees of freedom for the uncorrected abundance estimate, $\hat{N}_S$ (see Buckland et al., 1993).

The CV of combined abundance estimates ($\hat{N}_{Ui}$) was computed by

$$SE(total) = \sqrt{\left\{SE^2(\hat{N}_{U1}) + SE^2(\hat{N}_{U2}) + \ldots + SE^2(\hat{N}_{Ui})\right\}}, \quad (16)$$

and

$$CV(total) = \frac{SE(total)}{\hat{N}_U(total)}. \quad (17)$$

Results

The three line-transect surveys covered 2061 km of transect, and 231 sightings were used to estimate density (Table 2). Sighting rates were highest around Banks Peninsula (Table 3).

The simultaneous boat-and-helicopter surveys indicated that boat observers missed 11.4% of the dolphins on the trackline, but that strong responsive movement towards the boat resulted in apparent densities twice as high as they normally would be (Table 3). If the observers' attention was drawn to dolphin groups by the position of the helicopter, the results of these trials would be biased. This is unlikely, however, because several groups sighted by the helicopter observer subsequently passed within 200 m of the boat and were not seen by observers. We saw no evidence that the dolphins were affected by the helicopter.

Detection functions for boat-and-helicopter sightings (Fig. 5, C and D) are relatively smooth in comparison

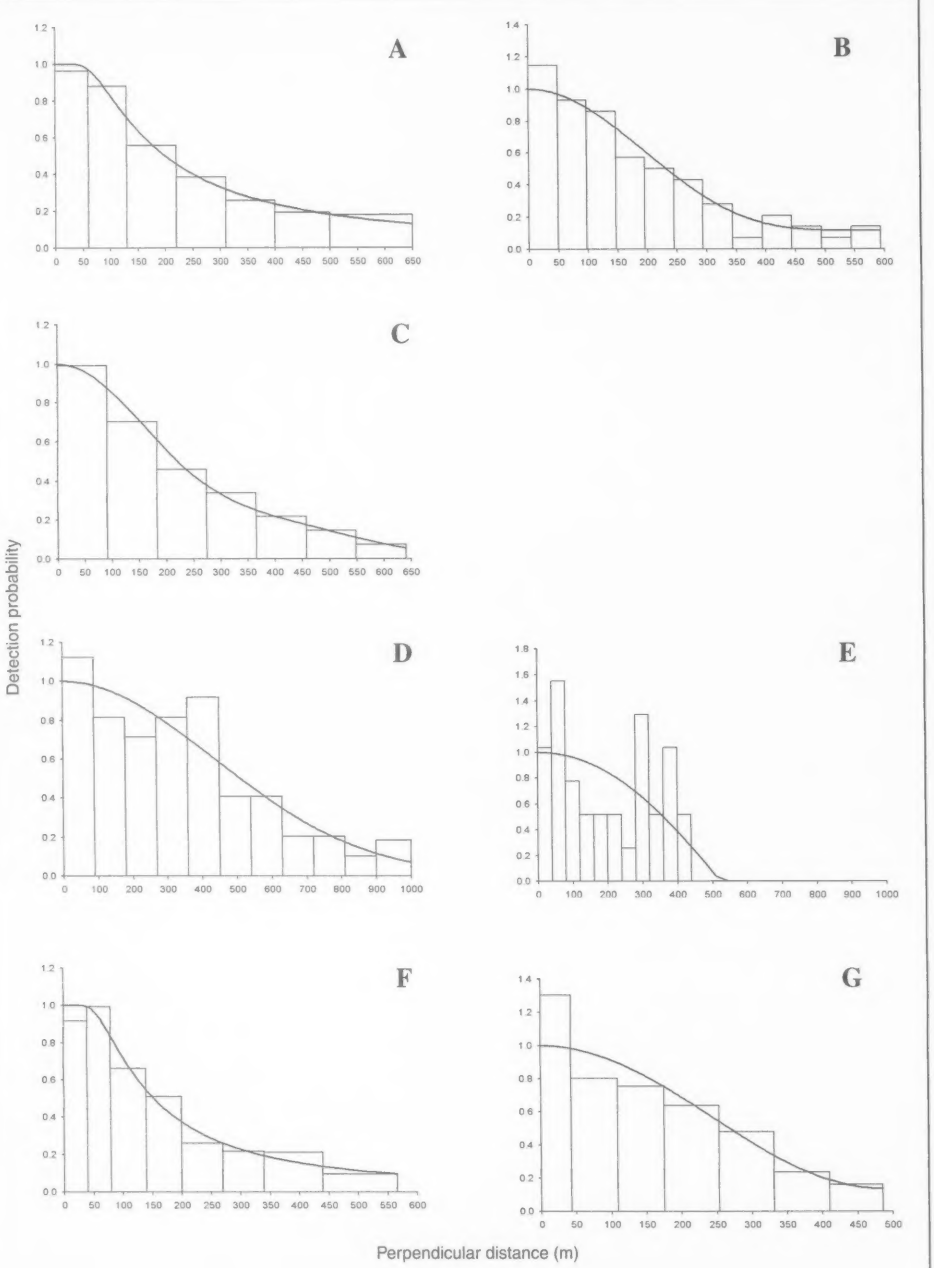

Figure 5

Histograms of perpendicular sighting distances, and their fitted detection functions as used to estimate effective strip width. n = number of sightings. The fitted model (hazard, cosine, uniform, or half normal) and any adjustments to it (cosine or none) are given in brackets. (**A**) 1997–98 harbors and bays (n=71; hazard/cosine); (**B**) 1997–98 open coasts (n=75; uniform/cosine); (**C**) 1998–99 open coasts (n=121; half-normal/cosine); (**D**) 1998–99 helicopter sightings (n=58; half-normal); (**E**) 1998–99 duplicate sightings (n=33; uniform/cosine); (**F**) 1999–2000 harbors and sounds (n=70; hazard/cosine); (**G**) 1999–2000 open coasts (n=89; uniform/cosine).

with those presented in Turnock et al. (1995). The detection function for the duplicate sightings (Fig. 5E) was more difficult to fit. Given the restricted sample size of duplicates (n=33), this result is not unexpected.

In the 1998–99 Timaru to Long Point and 1999–2000 Motunau to Farewell Spit surveys, robust estimation of ESW was facilitated by addition of extra sightings gained under similar sighting conditions at Banks Peninsula (Fig. 5, C, F, G). None of the three surveys showed significant evidence of larger groups being seen farther away. A broad pattern of abundance declining to the north and south of the Timaru–Banks Peninsula area is evident (Fig 2, Table 2). We made six sightings on 288 km of offshore lines (4–10 nmi offshore), confirming that densities in this zone are low.

Information on sea state is usually collected during boat line-transect surveys and sometimes used to poststratify data (e.g., Barlow, 1995). In our study this was not advantageous, for three reasons. 1) We avoided collecting data in conditions with whitecaps; therefore only a few sightings were collected in Beaufort 3. Hence variance estimates for this Beaufort state are large. 2) Differences among Beaufort states for key parameters such as sighting rate, average group size, and effective strip width were small and showed overlapping confidence intervals (we concede that statistical power is low because of reason 1 stated above). Note that data were pooled in the same way as for ESW estimation. 3) Stratification by Beaufort state does not produce abundance estimates that match the zones of intrinsic management interest (e.g., Banks Peninsula Marine Mammal Sanctuary; Dawson and Slooten, 1993).

Discussion

The catamaran survey platform was a near-ideal vessel for close inshore surveys. The sighting platform (Fig. 1) was a relatively inexpensive modification (~US$2000) that could be dismantled in about 10 minutes to allow sailing. The vessel's minimal draught allowed coverage of very shallow areas, which are an important part of the distribution of Hector's dolphin and many other inshore cetaceans. Although catamarans are inherently resistant to rolling, pitching can be a problem when motoring into a head sea or swell. We minimized this pitching by arranging lines so they could be run down-swell. The 45° placement of lines facilitated this reduction in pitching because it provided two alternative sets of lines (at 90° to one another). Further, these could be run inshore or offshore, allowing a choice of four options.

A significant advantage of vessels with low running costs is that the cost of training is low. We could afford to spend 7–10 days training before each survey. Further, waiting for weather to improve is inexpensive; therefore one does not need to gather data in marginal sighting conditions.

Estimated abundances (Table 4) were not significantly different from those estimated in the 1984–85 strip transect survey. Recent mark-recapture estimates of dolphin abundance at Banks Peninsula in 1996, based on photo-ID data, differed from the line-transect estimate for this area by less than 6% (Gormley, 2002; Jolly-Seber model allowing different capture probabilities between first and subsequent captures).

Our surveys confirmed previous work showing the patchy nature of Hector's dolphin distribution (Dawson and Slooten, 1988). Research at Banks Peninsula on the alongshore range of individually identified dolphins has shown a mean alongshore range of about 31 km (SE=2.43; Bräger et al., 2002). Despite wide-ranging surveys over 13 years, the most extreme sightings of any individual were 106 km apart. These data indicate very high site fidelity and indicate that even small-scale discontinuities in distribution may be long lasting. Lack of extensive movement along-shore, and hence limited contact with neighboring populations, is likely to be the mechanism by which Hector's dolphin has become segregated into genetically distinct populations (Pichler et al., 1998; Pichler and Baker, 2000).

The new abundance data, in combination with the genetic data indicating segregation of Hector's dolphin into four populations (Pichler and Baker, 2000) and modeling work indicating that the species is in decline in most of its range owing to bycatch in gill nets (Martien et al., 1999; Slooten et al., 2000), underscore the urgent need for better information on bycatch rates.

Despite strong evidence of bycatch throughout the species' range, observer coverage sufficient to estimate bycatch has been achieved only in one area (Canterbury) for one fishing season (1997–98; Baird and Bradford, 2000). During this season six Hector's dolphins were observed entangled in commercial gill nets (a further two were caught but released alive), resulting in a bycatch estimate of 17 individuals (Starr[3]). One mortality was observed in a trawl net, but very low observer coverage prevented any calculations of overall trawl bycatch (Baird and Bradford, 2000). No attempt was made to assess bycatch in recreational gillnetting during this period, but during a more recent summer (2000–01) five Hector's dolphin mortalities occurred in gill nets that were probably set by recreational fishermen (Department of Conservation and Ministry of Fisheries, 2001). It is not reasonable to assume that all mortalities in recreational gillnets are detected. In our opinion it is likely that combined commercial and recreational gillnet bycatch off Canterbury is about 15–30 individuals per year.

Hector's dolphin abundance on the north, east, and south coasts of the South Island estimated from the surveys reported in the present study is 1880 individuals (CV=15.7%). Hector's dolphins are more common on the

[3] Starr, P. 2000. Comments on "Estimation of the total bycatch of Hector's dolphins (*Cephalorhynchus hectori*) from the inshore trawl and setnet fisheries off the east coast of the South Island in the 1997–98 fishing year." Unpublished paper presented to Conservation Services Levy Working Group, 28 p. Department of Conservation, P.O. Box 10-420 Wellington, New Zealand.

Table 4

Corrected abundance estimates (only strata with sightings are listed). Number of sightings represents only those made in that stratum and used for density estimation. The number used for estimating effective half strip width.(ESW) differs because it includes sightings from extra transects in areas of similar exposure and transects repeated on the same day (and hence not true replicates for the purposes of estimating density).

Survey zone	Stratum	No. of sightings	ESW (m) (n, CV%)	$N_{(c)}$ (CV%)	Lower 95% CI	Upper 95% CI
Motunau to Timaru (1997–98)	Akaroa harbor	56	275 (71, 22.6)	62 (33.9)	32	121
	Other harbors and bays	8	275 (71, 22.6)	14 (67.5)	3	79
	Banks Peninsula Marine Mammal Sanctuary (BPMMS) (excluding harbors and bays)	62	261 (75, 10.3)	821 (22.1)	535	1258
	<4 nmi offshore, to the north and south of BPMMS	19	261 (75, 10.3)	300 (36.5)	133	679
Timaru to Long Point (1998–99)	Timaru–Long Point (excluding Te Waewae Bay)	13	268 (121, 10.5)	310 (28.4)	201	478
	Te Waewae Bay	14	268 (121, 10.5)	89 (32.4)	36	218
Motunau to Farewell Spit (1999–2000)	Queen Charlotte Sound	3	214 (70, 20.2)	20 (100.5)	4	110
	Cloudy and Clifford Bay	13	277 (89, 6.1)	162 (55.4)	56	474
	Cape Campbell–Motunau	5	277 (89, 6.1)	102 (55.2)	34	305
Total				1880 (21.3)	1246	2843

South Island west coast, where an aerial survey of similar design resulted in an estimate of 5388 (CV=20.6%; Slooten et al., in press). Thus Hector's dolphin abundance in South Island waters is estimated at 7268 individuals (CV=15.8%). The North Island subspecies of Hector's dolphin, now considered critically endangered (IUCN[4]) remains to be surveyed quantitatively.

The new abundance estimates provide an empirical basis from which to calculate levels of take that would still allow the currently depleted populations to recover (e.g., Wade, 1998). These levels of take should be seen as short-term targets for bycatch reduction in gill and trawl nets. For the management of Hector's dolphin to be put on a rational basis, a more comprehensive and wide-ranging assessment of bycatch, including statistically robust observer programs in coastal fisheries, is urgently needed.

Acknowledgments

These surveys were funded principally by Department of Conservation Contracts SCO 3072, 3074, and 3075. Significant contributions to equipment used in the survey were made by the New Zealand Whale and Dolphin Trust and the University of Otago. We are very grateful for the hard work put in by the other observers: Laszlo Kiss, Nadja Schneyer, Gail Dickie, Niki Alcock, Lesley Douglas, James Holborow, Ellie Dickson, Guen Jones, Will Rayment, and Dan Cairney. Jay Barlow, Jeff Laake, Anne York, and Debbie Palka shared their thoughts on survey design and field methods. David Fletcher helped with aspects of variance estimation. Akaroa Harbour Cruises provided much appreciated field support. Daryl Coup wrote the sightings program we used to collect data in the field. Otago University's Department of Surveying very helpfully provided the GPS base-station data for postprocessing our GPS fixes.

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Description and growth of larval and pelagic juvenile pygmy rockfish (*Sebastes wilsoni*) (family Sebastidae)

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Abstract—A developmental series of larval and pelagic juvenile pygmy rockfish (*Sebastes wilsoni*) from central California is illustrated and described. *Sebastes wilsoni* is a noncommercially, but ecologically, important rockfish, and the ability to differentiate its young stages will aid researchers in population abundance studies. Pigment patterns, meristic characters, morphometric measurements, and head spination were recorded from specimens that ranged from 8.1 to 34.4 mm in standard length. Larvae were identified initially by meristic characters and the absence of ventral and lateral midline pigment. Pelagic juveniles developed a prominent pigment pattern of three body bars that did not extend to the ventral surface. Species identification was confirmed subsequently by using mitochondrial sequence data of four representative specimens of various sizes. As determined from the examination of otoliths, the growth rate of larval and pelagic juvenile pygmy rockfish was 0.28 mm/day, which is relatively slow in comparison to the growth rate of other species of *Sebastes*. These data will aid researchers in determining species abundance.

Manuscript submitted 9 June 2003 to Scientific Editor's Office.

Manuscript approved for publication 25 February 2004 by the Scientific Editor.

Fish. Bull. 102:452–463 (2004).

Rockfishes (genus *Sebastes*) form a diverse group comprising at least 72 species occurring in the northeastern Pacific (Love et al., 2002). Many of these species represent a substantial portion of the groundfish fishery off the west coast of North America, accounting for 20% of the groundfish landings in California in 2000 (Pacific Fishery Management Council, 2000). A few species are relatively abundant but are not harvested because of their small size. These species play vital roles in the community ecology, including providing prey for the larger, commercially important species. The pygmy rockfish (*Sebastes wilsoni*) having a maximum size of 23 cm total length, is among these small species (Love et al., 2002). Pygmy rockfish are common over sediment and rocky seafloor habitats at a depth of 30–274 m (Stein et al., 1992; Yoklavich et al., 2000). Stein et al. (1992) observed that pygmy rockfish were by far the most abundant fish species off Heceta Bank, Oregon, and Love et al. (1996) reported "clouds" of pygmy rockfish mixed with two other small species, squarespot rockfish (*S. hopkinsi*) and halfbanded rockfish (*S. semicinctus*) off southern California. In Soquel Canyon in central California, pygmy rockfish dominated fish assemblages in rock-boulder habitat at 75–175 m (Yoklavich et al., 2000).

Accurate identification of larval stages is critical. Biomass of rockfish populations can be estimated from larval production (Ralston et al., 2003) and larval and juvenile abundance studies (Moser and Butler, 1987; Hunter and Lo, 1993). If the larval and juvenile rockfish analyzed in these studies are not correctly identified, it could lead to either over- or underestimates of biomass or recruitment potential of a population. Identification of young stages of *Sebastes* has been accomplished through rearing studies and through descriptions based on developmental series of field-caught specimens of various sizes (Matarese et al., 1989; Moser, 1996). Otolith morphologies have been useful in discerning some *Sebastes* species (Laidig and Ralston, 1995; Stransky, 2001). Recently, molecular methods have proven to be an effective tool for the identification of *Sebastes* larvae (Seeb and Kendall, 1991; Rocha-Olivares, 1998; Rocha-Olivares et al., 2000).

In this study, we identify and describe the larvae and pelagic juveniles of pygmy rockfish based on morphometrics and pigmentation patterns, and estimate age and growth at two

developmental stages. Further, we examine otolith radius at time of larval extrusion to separate pygmy rockfish from other similarly pigmented *Sebastes* specimens. We also use mitochondrial DNA (mtDNA) sequence data to identify four putative pygmy rockfish specimens representing a continuum of late-larval through pelagic juvenile stages. The molecular results are used to confirm identifications based on morphological, meristic, and pigmentation characters and to assure that the assembled developmental series is monospecific.

Methods

Specimen collection

Specimens of larval and pelagic juvenile pygmy rockfish were obtained from research cruises conducted aboard the NOAA RV *David Starr Jordan* off central California. Specimens were collected in midwater (5–30 m) from mid-May to mid-June, 1990–92, between Bodega Bay (north of San Francisco) and Cypress Point (south of Monterey Bay) by using a 26 m×26 m modified Cobb midwater trawl (12.7-mm stretched-mesh codend liner). Specimens also were collected during early March, 1992–93, between Salt Point (north of San Francisco) and Cypress Point with a 5 m×5 m modified Isaacs-Kidd (MIK) frame trawl with 2-mm net mesh and 0.505-mm mesh codend. Specimens from the Cobb trawl were frozen and specimens from the MIK frame trawl were preserved in 95% ethanol for later analysis.

Meristics, morphometrics, and body pigmentation

We examined pigmentation patterns and physical characteristics of 122 pygmy rockfish larvae and pelagic juveniles. Standard length (SL) was measured for each individual and sizes ranged from 8.1 to 34.4 mm. Specimens greater than 19.9 mm were identified by using meristic characters (Chen, 1986; Matarese et al., 1989; Moreland and Reilly, 1991; and Laroche[1]), and pigment patterns were recorded. Specimens less than 20 mm were identified initially from pigment patterns from a series starting with the smallest (8.1 mm SL) identifiable individuals with complete fin-ray counts. Counts of dorsal-, anal-, and pectoral-fin rays, and the number of gill rakers on the first arch were recorded whenever possible and subsequently used in identifications. Gill raker counts were obtained only from fish larger than 15 mm SL.

We measured snout-to-anus length, head length, snout length, eye diameter, body depth at the pectoral fin base, body depth at anus, and pectoral-fin length on 16 specimens ranging from 8.1 to 29.6 mm SL, following Richardson and Laroche (1979). Head spination was examined on thirty-three specimens (8.1 to 29.6 mm SL) that were stained with alizarin red-s. Terminology for head spination follows Richardson and Laroche (1979). In the following descriptions, larval and juvenile lengths always refer to SL and pigmentation always refers to melanin.

Otolith examination

Sagittal otoliths were removed from 61 larval and pelagic juvenile pygmy rockfish (8.1–34.4 mm SL), and growth increments were counted beginning at the first increment after the extrusion check (the mark in the otolith formed when the larvae are released from their mother) by using a compound microscope at 1000× magnification (see Laidig et al., 1991). No validation of the these growth increments was performed during the present study, and none has been conducted by other researchers. However, we assumed that these growth increment counts corresponded to daily ages based on validation of daily growth increments in other co-occurring rockfishes, namely shortbelly rockfish, *S. jordani* (Laidig et al., 1991), black rockfish, *S. melanops* (Yoklavich and Boehlert, 1987), bocaccio, *S. paucispinis*, chilipepper, *S. goodei*, widow rockfish, *S. entomelas*, and yellowtail rockfish, *S. flavidus* (Woodbury and Ralston, 1991). The radius of the otolith was measured from the primordium to the postrostral edge of the extrusion check for comparison with similar measurements from other *Sebastes* spp. (as reported in Laidig and Ralston, 1995). Transformation from the larval stage to the pelagic juvenile stage was ascertained by the presence of accessory primordia (Laidig et al., 1991; Lee and Kim, 2000).

Molecular confirmation

Total genomic DNA was isolated from skeletal muscle tissue of four larval and juvenile putative pygmy rockfish specimens by using a CTAB and phenol-chloroform-isoamyl alcohol protocol (Winnepenninckx et al., 1993; Hillis et al., 1996). These four specimens ranged in length from 15 to 27 mm and had pigment patterns similar to the fish identified as pygmy rockfish in the present tudy. Polymerase chain reaction (PCR) amplifications and sequencing of partial mitochondrial DNA regions (cytochrome *b* [cyt-*b*] and control region [CR]) followed the methods of Rocha-Olivares et al. (1999a, 1999b). PCR products were verified on 2% agarose gels and purified by using a QIAquick™ PCR Cleanup Kit (Qiagen, Inc., Valencia, CA) following manufacturer protocols. Complementary strand sequence data were generated by using ABI PRISM™ DyeDeoxy™ terminator cycle sequence chemistry on an automated sequencer (Applied Biosystems, Model 377, Foster City, CA).

Cytochrome *b* sequence data (750 base pairs) from the four specimens were aligned with (previously generated) orthologous sequences from 119 individuals representing 61 species of *Sebastes* (Rocha-Olivares et al., 1999b). Species identifications, based on cyt-*b* data, were made by using distance-based cluster analyses in PAUP v4.0b2 (Phylogenetic Analysis Using Parsimony,

[1] Laroche, W. A. 1987. Guide to larval and juvenile rockfishes (*Sebastes*) of North America. Unpubl. manuscript, 311 p. Box 216, Enosburg Falls, VT 05450.

version 4, Sunderland, MA) and pairwise comparisons of sequence divergence (i.e., the number of nucleotide differences between two individuals expressed as a percentage). A secondary data set, which included an additional 450 base pairs of control region sequence, was generated for the four undetermined specimens and for a subgroup of known reference species with low levels of sequence divergence from the four putative pygmy rockfish specimens (Puget Sound rockfish [*S. emphaeus*], redstripe rockfish [*S. proriger*], harlequin rockfish [*S. variegatus*], sharpchin rockfish [*S. zacentrus*], and pygmy rockfish). Species identifications, based on this extended (cyt-*b* + CR) data subset, followed analyses described above.

Table 1

Frequency of occurrence (number of fish) of dorsal-, anal-, and pectoral-fin ray counts, and gill raker counts from 122 pygmy rockfish (*Sebastes wilsoni*).

Character	Count	Frequency of occurrence	Percent occurrence
Dorsal-fin rays	12	8	7
	13	110	91
	14	3	2
Anal-fin rays	5	2	2
	6	116	95
	7	4	3
Pectoral-fin rays	16	5	5
	17	92	90
	18	5	5
Gill rakers	36	11	14
	37	15	18
	38	22	28
	39	20	25
	40	10	13
	41	1	1
	42	1	1

Results

General development

All 122 fish had completed notochord flexion and possessed a full complement of segmented fin rays by 8.1 mm. The mode for dorsal-fin ray counts was 13, for anal-fin rays 6, and for pectoral-fin rays 17 (Table 1). The mode for gill raker counts was 38, and the range was 36–42. Anal- and dorsal-fin spines began to develop between 9.1 and 14.0 mm. Lateral line pores began to develop at 29 mm, although a full complement (37 to 46 pores) was not reached in our specimens. Morphometric measurements were taken from 16 individual pygmy rockfish of 8.1–29.6 mm (Table 2).

Head spination

At 8.1 mm, the postocular, parietal, nuchal, inferior posttemporal, supracleithral, superior opercular, preopercular (with the exception of the 2nd anterior), and 1st and

Table 2

Morphometric measurements (in mm) from 16 individuals of pygmy rockfish (*Sebastes wilsoni*).

SL	Snout–anus length	Head length	Snout length	Eye diameter	Body depth at pectoral base	Body depth at anus	Pectoral-fin length
8.1	5.2	3.3	0.8	1.3	2.8	2.3	1.5
9.0	5.3	3.0	1.0	1.5	3.0	2.3	1.7
10.8	6.5	4.2	1.2	1.7	3.5	2.8	2.3
12.1	7.0	4.3	1.3	2.0	3.8	2.8	2.5
12.8	7.7	4.8	1.3	2.2	3.7	3.0	2.5
14.2	8.3	4.7	1.3	2.2	4.3	3.5	3.3
15.2	9.2	5.7	1.7	2.0	4.2	3.5	3.5
16.2	9.7	5.2	1.5	2.0	4.5	3.8	3.8
17.5	10.8	6.2	2.0	2.3	5.0	3.8	4.2
18.6	11.5	6.0	2.0	2.7	5.5	4.3	5.0
20.7	12.7	6.2	2.0	2.7	6.2	5.2	5.0
22.3	14.2	6.7	2.2	2.8	6.8	5.7	6.2
23.8	14.5	6.7	2.0	3.2	7.3	6.3	5.9
24.3	14.2	7.0	2.2	2.7	7.0	5.8	6.3
28.9	17.0	8.0	2.6	3.3	8.5	7.3	7.0
29.6	16.9	7.8	2.6	3.5	8.7	7.5	6.9

2nd inferior and 1st superior infraorbital spines were present (Table 3). The nasal, pterotic, and 4th superior infraorbital first appeared at 10.8 mm. At 12.1 mm, the inferior opercular spine became evident. Between 14.2 and 17.5 mm, the preocular, tympanic, superior postemporal, 2nd anterior preopercular, and 3rd superior infraorbital spines formed. After 17.5 mm, no further changes in head spination were noted. The supraocular, coronal, 3rd inferior infraorbital, and 2nd superior infraorbital spines did not occur on any of the fish examined.

Body pigmentation

At 9.1 mm, pygmy rockfish had no pigment along the lateral and ventral body surfaces (Fig. 1A, Table 4). Pigment was heavy on the top of the head and present on the operculum. The dorsal midline surface had a few melanophores under the soft dorsal fin. The anterior lower jaw was pigmented on the tip and one to two melanophores were present on each side of the snout near the tip. Pigment also was present at the base and on the distal half of the pectoral fin.

By 14.0 mm, dense pigment along the dorsal midline stretched from the caudal peduncle to the first dorsal-fin spine (Fig. 1B, Table 4). The only lateral pigment on the body consisted of a few melanophores along the midline near the caudal peduncle. The ventral surface, including the anal and pelvic fins, remained unpigmented. Pigment on the pectoral fins had mostly disappeared by 11.0 mm and was rarely observed at 14.0 mm. Opercular and head pigment increased in density by 14.0 mm. Pigment along the lower edge of the orbit began to

Table 3

Development of head spines in individual pygmy rockfish (*Sebastes wilsoni*). "1" means spines present and "0" means spines absent.

	Standard length (mm)															
Spines	8.1	9.0	10.8	12.1	12.8	14.2	15.2	16.2	17.5	18.6	20.7	22.3	23.8	24.3	28.9	29.6
Nasal	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Preocular	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
Supraocular	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Postocular	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coronal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tympanic	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
Parietal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Nuchal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pterotic	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Posttemporal																
Superior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
Inferior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Supracleithral	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Opercular																
Superior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Inferior	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	
Preopercular																
1st anterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2nd anterior	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
3rd anterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1st posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2nd posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3rd posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4th posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5th posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Infraorbital																
1st inferior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2nd inferior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3rd inferior	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1st superior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2nd superior	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3rd superior	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
4th superior	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1

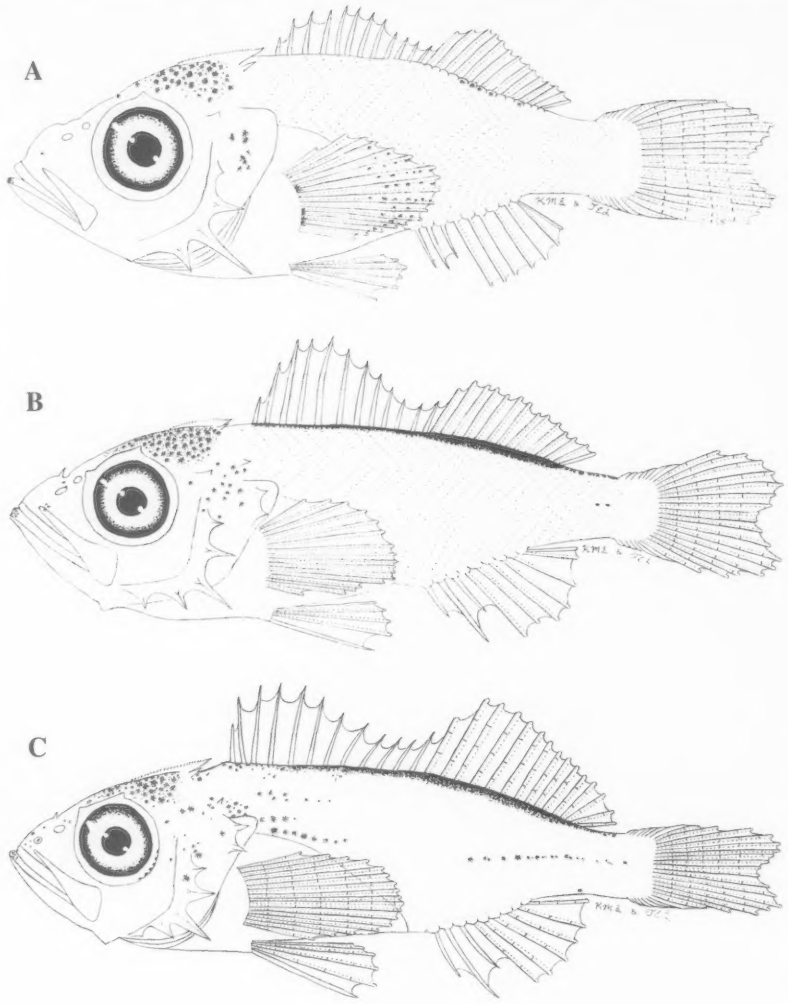

Figure 1

Developmental series of pygmy rockfish (*Sebastes wilsoni*) (drawn by authors). (**A**) 9.1-mm-SL larva; (**B**) 14.0-mm-SL larva; (**C**) 17.5-mm-SL larva; (**D**) 23.0-mm-SL pelagic juvenile; (**E**) 28.6-mm-SL pelagic juvenile. Arrow marks ventral end of anterior body bar; (**F**) 34.4-mm-SL pelagic juvenile. Note that not all head spines are included in the illustrations.

form in some specimens by 12.0 mm and was visible in most specimens by 14.0 mm (Fig. 1B). Snout pigment was represented by one or four melanophores. Anterior lower jaw pigment was heavy and confined to the tip of the jaw.

By 17.5 mm, the dorsal midline pigment had become much darker and denser (Fig. 1C, Table 4) and extended from the caudal fin to the head region, except for a gap where the nape pigment was beginning to form. All fins were unpigmented. Pigment along the ventral body midline began to form at 17.5 mm, with a few postanal melanophores. Lateral midline pigment formed in two locations. Melanophores near the peduncle increased anteriorly, and pigment began forming dorsal to the gut cavity and increased posteriorly toward the peduncle. A body bar began to form on the lateral surface above the pectoral fin between the spinous dorsal fin and the anterior lateral midline pigment. Opercular, eye, and head pigment all increased in density. Melanophores on the snout also became more prevalent between the tip of

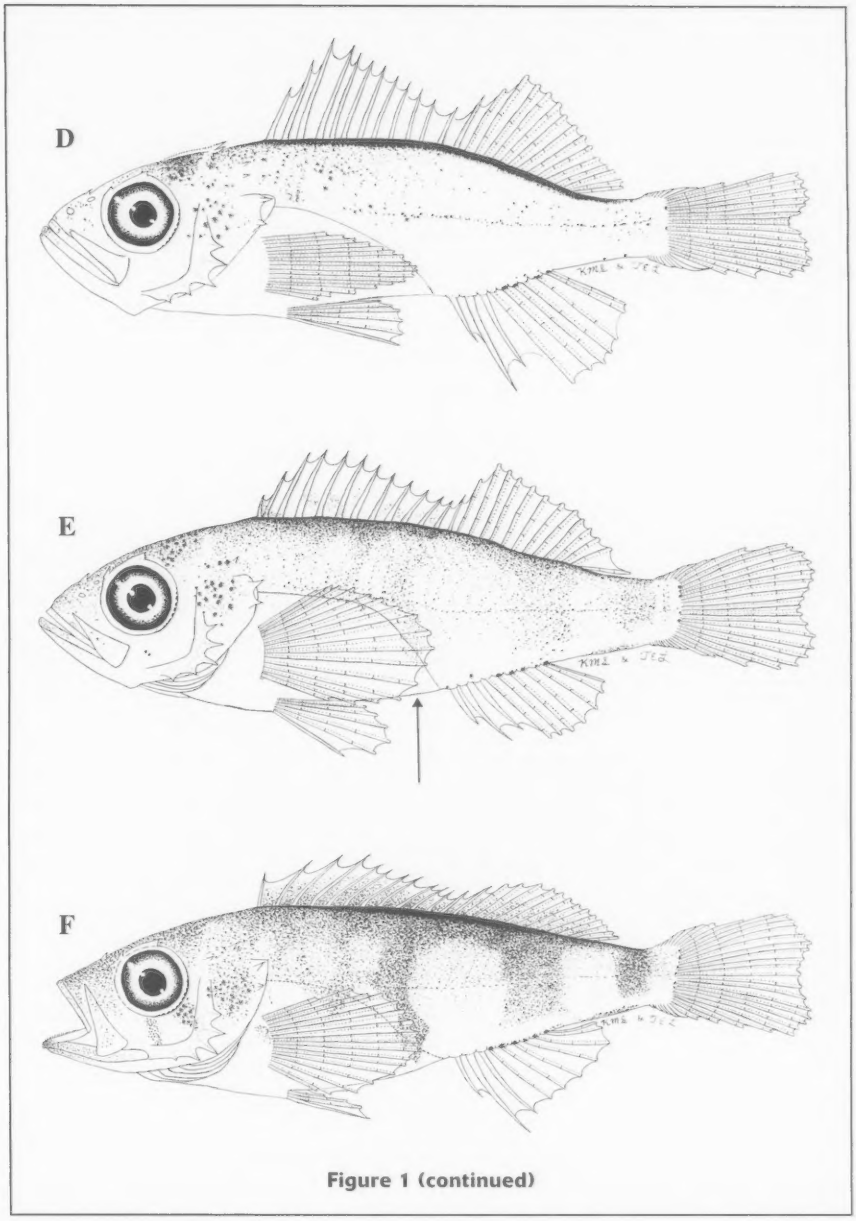

Figure 1 (continued)

the upper jaw and top of the head. Pigment on the tip of the lower jaw spread posteriorly and became denser than in smaller specimens.

When the pelagic juveniles reached 23.0 mm, the dorsal midline pigment was a dark strip extending from the caudal fin to the head (Fig. 1D, Table 4). Nape pigment almost merged with the head pigment, except for a small unpigmented area below the insertion of the parietal and nuchal spines. Hypural pigment was present distally in all individuals at this size and at larger sizes (n=30). Anterior and posterior lateral midline pigment merged to form a continuous line along the body. The number of melanophores increased on the ventral body surface, and a few melanophores were present at the anal-fin ray bases and along the ventral midbody posterior to the anal fin. The anterior body bar broadened and became more defined. A few melanophores on the flanks under the soft dorsal fin began to form a midbody bar. A third body bar began to form on the caudal peduncle. Pigment on the operculum, top of the

Table 4

Proportion of pygmy rockfish (*Sebastes wilsoni*) with melanophores present at various areas averaged over 1.0-mm size bins (range of ±1.0 mm). SL = standard length in mm. LJ = anterior tip of the lower jaw, EYE = posterior-ventral edge of the eye orbit, SNOUT = dorsal surface anterior to the eyes, OPER = operculum, CHK = radiating cheek bars, NAPE = nape pigment, DORS = dorsal body surface, VENT = ventral body surface, MID = along the lateral midline, HYP = hypural region, DFIN = spinous dorsal fin, PEC = blade of the pectoral fin, CAUD = caudal body bar, ANT = anterior body bar, MIDB = midbody bar, CANAL = clear area dorsal to anal fin, MAX = pigment on the side of the maxilla, UND = pigment under lower jaw.

SL	N	LJ	EYE	SNOUT	OPER	CHK	NAPE	DORS	VENT	MID	HYP	DFIN	PEC	CAUD	ANT	MIDB	CANAL	MAX	UND
8	2	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
9	3	1.0	0.0	1.0	1.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
10	6	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
11	10	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0
12	5	1.0	0.2	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
13	7	1.0	0.1	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
14	8	1.0	0.9	1.0	1.0	0.0	0.0	1.0	0.3	0.5	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
15	6	1.0	0.8	1.0	1.0	0.0	0.0	1.0	0.3	0.7	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
16	7	1.0	0.9	1.0	1.0	0.0	0.0	1.0	0.1	0.9	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
17	8	1.0	0.8	1.0	1.0	0.0	0.3	1.0	0.3	0.8	0.4	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
18	14	1.0	1.0	1.0	1.0	0.0	0.8	1.0	0.6	1.0	0.7	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0
19	7	1.0	1.0	1.0	1.0	0.0	0.6	1.0	0.3	1.0	0.3	0.0	0.0	0.1	0.0	0.7	0.0	0.0	0.0
20	2	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.5	0.0	1.0	0.0	0.0	0.0
21	6	1.0	1.0	1.0	1.0	0.0	0.8	1.0	0.8	1.0	0.5	0.0	0.0	0.3	0.3	0.7	0.0	0.0	0.0
22	4	1.0	1.0	1.0	1.0	0.0	1.0	1.0	0.8	1.0	0.5	0.0	0.0	0.5	0.3	1.0	0.0	0.0	0.0
23	1	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
24	5	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	0.8	1.0	0.2	0.0	0.0
25	1	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
26	1	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0
27	2	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0
28	3	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	0.7	0.7
29	6	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	0.3	1.0	1.0	1.0	1.0	0.7	1.0
30	3	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0
31	3	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0
32	3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
33	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
34	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

head, and snout also increased in density, and pigment formed posteriorly along the upper and lower jaws. Melanophores posteriorly around the eye socket increased in number. The fins remained unpigmented.

Pygmy rockfish 28.6 mm long had dorsal pigment that stretched continuously from the jaws to the caudal fin (Fig. 1E, Table 4). Pigmentation was heavy along the dorsal midline, head, and nape. Snout pigmentation also intensified. More melanophores were present on the hypural margin. Pigment along the ventral body surface darkened, especially in the area posterior to the anal fin. More melanophores were observed at the anal-fin ray articulations than on smaller specimens. The three body bars increased in width and length and were better defined than on smaller specimens. The bar on the caudal peduncle began to exhibit a rectangular shape that is characteristic of the juvenile stage. The midbody bar also took on a rectangular shape, although the dorsal half was indented. The midbody bar and the caudal peduncle bars did not reach the ventral midline. The anterior body bar extended from the spinous dorsal fin to the vent (see arrow Fig. 1E). Anteriorly, the bar formed a more or less rectangular pattern on the dorsal half of the body above the pectoral fin. In general, the lateral body surface became more heavily pigmented, especially on the dorsal half. The lateral midbody pigment line began to be incorporated into the body bars. Opercular pigment became denser and merged with the nape pigment. The area anterior to the nape and operculum was less pigmented than the surrounding areas. Pigment along the posteroventral portion of the orbit became denser than in smaller specimens. A cheek bar began to form ventral to the eye (as evidenced by the two melanophores in Fig. 1E). Melanophores formed along the ventral surface of the lower jaw and covered the lateral surface of the upper jaw. Pigment began to develop on the membranes of the spinous dorsal fin, typically with some unpigmented areas between the dorsal fin pigment and the dorsal body pigment.

The largest specimen, 34.4 mm, had the densest and most distinctive pigmentation (Fig. 1F, Table 4). Pigment was present on most of the body. Along the dorsal surface, the pigment formed a complete line from the tip of the upper jaw to the caudal fin. The number of melanophores increased along the hypural region, the postanal ventral midline, and at the anal-fin articulations. The mid- and caudal body bars were rectangular and still did not reach the ventral midline, leaving an unpigmented ventrolateral area. The anterior body bar comprised heavy pigment extending posteriorly between dorsal-fin spines VIII–XI and the vent, a lighter area just anterior to this, and another heavily pigmented area stretching from about dorsal-fin spines III–VI almost to the middle of the gut cavity. Anterior to this bar was an area of mottled pigmentation. Pigment was visible just anterior to the base of the pectoral fin. Pigment covered both the spinous and soft dorsal fins, except along the distal edge. All other fins remained unpigmented. Opercular pigment was dense and merged with the nape pigment, but these were separated from the head and eye pigment by an area of low pigment density. Two cheek bars radiated from the lower margin of the orbit. Pigment occurred along both jaws and covered the snout and ventral portion of the lower jaw.

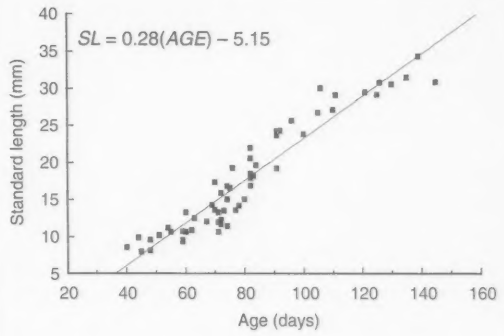

Figure 2

Standard length and age of pygmy rockfish (*Sebastes wilsoni*) (n=60). Solid line indicates predicted values from linear model.

Otolith examination

A linear relationship between standard length and age (as estimated from otolith increment counts) resulted in a good estimate of growth of pygmy rockfish (slope=0.28 mm/d; intercept=–5.15 mm; r^2=0.91; n=60; Fig. 2). The radius of the extrusion check ranged from 9.5 to 11.0 μm, averaging 10.5 μm (SD=0.29; n=60). Accessory primordia first appeared in a 19.8-mm specimen and were observed in otoliths from all larger specimens. Based on this character, transition from larval to pelagic juvenile stage occurs at around 20 mm SL.

Molecular confirmation

Interspecific levels of divergence, calculated among adult reference species, ranged from 0.13% (rougheye rockfish [*S. aleutianus*] vs. shortraker rockfish [*S. borealis*]) to 9.7% (black rockfish [*S. inermis*] vs. bocaccio) with an average of 4.1%. Two of the specimens (FT2 and FT3; Fig. 3A) were identical to one of the adult pygmy rockfish references (i.e. 0% sequence divergence) and differed from the other verified adult pygmy rockfish by a single nucleotide substitution (0.13% seq. div.). The remaining two specimens (FT1 and FT4; Fig. 3A) also were most similar to both adult pygmy rockfish references (0.13–0.40% seq. div.).

Although all four specimens were most similar to pygmy rockfish based on cytochrome *b* data, only a small number of nucleotide differences separated them from Puget Sound, redstripe, harlequin, and sharpchin rockfish (0.27–1.87%; Fig. 3A). A secondary data subset that included control region sequence (cyt-*b*+CR) yielded concordant results; all four larval specimens were

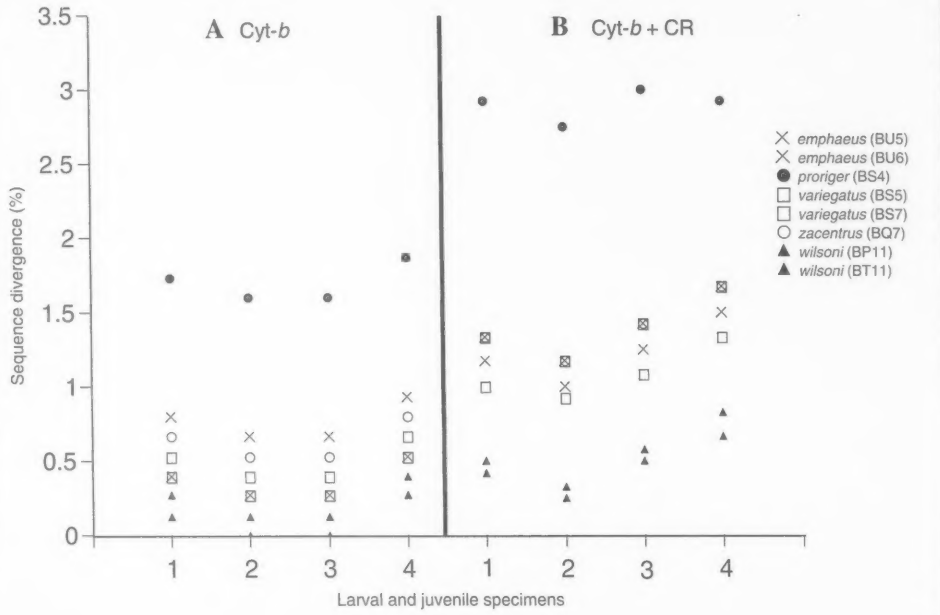

Figure 3

Percent sequence divergence based on the number of nucleotide differences in (**A**) cytochome *b* and (**B**) cytochrome *b* + control region between the four putative pygmy rockfish (*Sebastes wilsoni*) specimens (FT1–FT4) and five closely related reference species of *Sebastes*, including two adult *S. wilsoni*. Percent sequence divergence was calculated as the number of nucleotide differences over 750 base pairs (cyt-*b*) and 1200 base pairs (cyt-*b*+CR).

most similar to pygmy rockfish (0.25–0.83%; Fig. 3B). Increased levels of interspecific nucleotide variation, attributable to the faster evolving control region, resulted in more pronounced differences between the four specimens and the other species of *Sebastes* within the subset (range: 0.83–3.00%; Fig. 3B). Additionally, a distance-based analysis (UPGMA) of haplotypes (cyt-*b*+CR) clustered all four specimens with pygmy rockfish reference material.

Discussion

Postflexion larval pygmy rockfish can be identified through a combination of pigment and meristic characters. At approximately 8–10 mm, the larval pigment pattern is similar to only four of the 30 *Sebastes* species illustrated in the literature that occur within our geographic area (Matarese et al., 1989; Moser, 1996; Laroche[1]): yellowtail (*S. flavidus*), blue (*S. mystinus*), canary (*S. pinniger*), and sharpchin rockfish. Yellowtail and blue rockfish can be separated from pygmy rockfish because they exhibit ventral body and hypural pigment at this size—pigment that does not show up in pygmy rockfish until approximately 14 and 15 mm, respectively. In canary rockfish, the presence of ventral body pigment and dorsal midline pigment posterior to the soft dorsal fin (instead of at the base of the soft dorsal-fin rays as in pygmy rockfish) can help differentiate this species from pygmy rockfish. Pigmentation patterns of sharpchin rockfish are very similar to pygmy rockfish at 10 mm; however, sharpchin rockfish retain pigmented pelvic fins until 12.7 mm (Laroche and Richardson, 1981). Counts of anal-fin rays often can be used to differentiate these two species because pygmy rockfish have six rays and sharpchin have rockfish seven rays (Chen, 1986; Matarese et al., 1989; Moreland and Reilly, 1991; Laroche[1]). There is a small overlap in anal-fin ray counts (approximately 7%), and, because of this, 100% certainty of identification cannot be reached by anal-fin ray counts alone. Therefore, in order to increase confidence in identifications, a combination of pigmentation and fin-ray counts should be employed. After approximately 15 mm, a full complement of fin rays and gill rakers typically is present and can be used in combination with pigmentation patterns to differentiate pygmy rockfish from most other rockfish species. In these late-stage larvae, only three species (yellowtail, black (*S. melanops*), and blue rockfish) have a pigment pattern that could be confused with pygmy rockfish (Matarese et al., 1989; Moser, 1996; Laroche[1]),

but these patterns can be easily separated by using meristic characters.

Pelagic juvenile pygmy rockfish have a distinctive pigment pattern consisting of three body bars that can be used to discriminate this species from other *Sebastes* species. Yellowtail, halfbanded, and redstripe rockfish are the only species that have a similar three-barred pigment pattern (Matarese et al., 1989; Moser, 1996; Laroche[1]). Yellowtail rockfish can be distinguished by the lack of cheek bars and the presence of body bars that extend all the way to the ventral surface. Also, in yellowtail rockfish, the body bars form at a larger size than in pygmy rockfish. In halfbanded rockfish, the most anterior body bar is more densely pigmented than the other bars and typically forms a diamond shape. The caudal body bar is much wider and covers the entire peduncle. Redstripe rockfish are the most similar and are difficult to separate from pygmy rockfish by using pigmentation alone. However, these two species can be separated with greater than 90% certainty by using meristic counts. Pygmy rockfish have a mean anal-fin ray count of 6 (95% from the present study, and 93% from Laroche[1]), whereas redstripe rockfish have an average of 7 anal-fin rays (100% from Chen, 1986; 97% from Laroche[1]).

It should be noted that the only illustration of pygmy rockfish prior to our study was a 35.0-mm pelagic juvenile by Laroche,[1] which showed several pigment differences from our specimens of equivalent size. Laroche's illustrated specimen had only faint body barring, no cheek bars, and no ventral pigment, whereas all our specimens had prominent body barring, at least one cheek bar, and ventral pigment along the anal-fin articulations. At this time we cannot determine whether these differences were due to geographic variability in pigment patterns (Laroche's specimen probably was collected farther north than all of our specimens), or a misidentification of the original specimen illustrated by Laroche.[1]

The identification of larval and pelagic juvenile pygmy rockfish used in our study was confirmed by using DNA sequence analyses. Previous molecular identifications and subsequent descriptions of juvenile starry rockfish (*S. constellatus*) and swordspine rockfish (*S. ensifer*) also were based on mitochondrial cytochrome *b* data (Rocha-Olivares et al., 2000). In our study, orthologous cytochrome *b* sequence was sufficient for identification purposes, particularly for those specimens exhibiting exact haplotype matches to reference adult pygmy rockfish (e.g., FT2/FT3: 0.0% sequence divergence). Relatively low levels of interspecific genetic variation occurred between larval specimens and several reference species (pygmy, sharpchin, harlequin, and Puget Sound rockfish, and, to a lesser extent, redstripe rockfish). Rocha-Olivares et al. (1999a) used control region sequence, in addition to cytochrome *b*, to resolve phylogenetic relationships among recently diverged species of the *Sebastes* subgenus *Sebastomus*. In the present study, the control region sequence was used to increase divergence levels between species and to aid in insuring correct molecular identifications of specimens FT1 and FT4. Species assignment to pygmy rockfish was supported by the smallest divergence (based on cyt-*b* and cyt-*b*+CR) from reference pygmy rockfish compared with the other *Sebastes* species.

Larval and juvenile pygmy rockfish can also be separated from other *Sebastes* species by comparing the radius of the extrusion check on their otoliths. Of the fourteen other *Sebastes* species or species complexes with measured otolith extrusion check radii (Laidig and Ralston, 1995; Laidig et al., 1996; Laidig and Sakuma, 1998), only four species and two complexes have radii close to the average extrusion check radii for pygmy rockfish (10.5 μm, SD=0.3). Stripetail rockfish (*S. saxicola*) had an average extrusion check radius (11.6 μm, SD=0.5) that was larger than the largest radius for pygmy rockfish (11.0 μm). Quillback rockfish (*S. maliger*) had an average extrusion check radius of 9.1 μm (SD=0.1), which was smaller than the smallest radius for pygmy rockfish (9.5 μm). Species with extrusion check radii similar to pygmy rockfish were kelp rockfish (*S. atrovirens*) at 10.6 μm (SD=0.2), blue rockfish at 10.9 μm (SD=1.1), and the copper rockfish (*S. caurinus*, extrusion check radius=10.5 μm; SD=0.4) and gopher rockfish (*S. carnatus*, extrusion check radius=10.6 μm; SD=0.3) complexes (see Laidig et al., 1996, for complex definitions). Of these species, the only one that would be confused with pygmy rockfish, by pigmentation alone, would be blue rockfish at small sizes. However, pygmy rockfish and blue rockfish are easily separated by using meristic characters.

Growth rates of larval rockfish generally are slow during the first month of life and increase thereafter (Laidig et al., 1991; Sakuma and Laidig, 1995; Laidig et al., 1996). Because the youngest fish in our study was estimated to be 40 days old, our linear model can not be used to estimate early larval growth rates. For pygmy rockfish older than 40 days, the growth rate of 0.28 mm/day was somewhat slower than that observed for other *Sebastes*. Woodbury and Ralston (1991) found that, for fish older than 40 days, growth rates varied from 0.30 for widow rockfish (*S. entomelas*) to 0.97 mm/day for bocaccio. Other species exhibiting slightly faster growth rates after 40 days of age include stripetail rockfish (0.37 mm/day; Laidig et al., 1996), grass rockfish (*S. rastrelliger*; 0.36 mm/day; Laidig and Sakuma, 1998), and shortbelly rockfish (*S. jordani*; 0.53 mm/day; Laidig et al., 1991). Yellowtail rockfish had a more similar growth rate, ranging from 0.19 to 0.46 mm/day (Woodbury and Ralston, 1991). These differences in growth may reflect genetic variability or responses to environmental variables. Woodbury and Ralston (1991) suggested that annual variability in growth rates of juvenile rockfish was related to year-to-year changes in environmental conditions, especially temperature. Boehlert (1981) determined that temperature greatly affected growth rate of young splitnose rockfish (*S. diploproa*) in the laboratory. Boehlert and Yoklavich (1983) observed slower growth rates for black rockfish in colder temperatures. Lenarz et al. (1991) analyzed the vertical

distribution of late larval and pelagic juvenile rockfish and determined that pygmy rockfish were present on average in deeper, colder water than that favored by other rockfish species. This spatial separation of pelagic juvenile pygmy rockfish and other *Sebastes* spp. may explain the slower growth observed in pygmy rockfish.

Acknowledgments

We would like to thank the scientists and crew from the Southwest Fisheries Science Center (SWFSC) who collected the samples aboard the NOAA RV *David Starr Jordan*. We thank Geoff Moser and Bill Watson (NOAA, SWFSC) for examining some of our pygmy rockfish specimens. Reference sequences of *Sebastes* were generated and kindly provided by personnel at the Fisheries Resources Division of the SWFSC, La Jolla, CA (R. D. Vetter, A. Rocha-Olivares, B. J. Eitner, C. A. Kimbrell, and C. Taylor). In addition, we thank Mary Yoklavich for all her valuable comments and all the reviewers who contributed to this manuscript.

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Estimating the emigration rate of fish stocks from marine sanctuaries using tag-recovery data

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Abstract—A critical process in assessing the impact of marine sanctuaries on fish stocks is the movement of fish out into surrounding fished areas. A method is presented for estimating the yearly rate of emigration of animals from a protected ("no-take") zone. Movement rates for exploited populations are usually inferred from tag-recovery studies, where tagged individuals are released into the sea at known locations and their location of recapture is reported by fishermen. There are three drawbacks, however, with this method of estimating movement rates: 1) if animals are tagged and released into both protected and fished areas, movement rates will be overestimated if the prohibition on recapturing tagged fish later from within the protected area is not made explicit; 2) the times of recapture are random; and 3) an unknown proportion of tagged animals are recaptured but not reported back to researchers. An estimation method is proposed which addresses these three drawbacks of tag-recovery data. An analytic formula and an associated double-hypergeometric likelihood method were derived. These two estimators of emigration rate were applied to tag recoveries from southern rock lobsters (*Jasus edwardsii*) released into a sanctuary and into its surrounding fished area in South Australia.

Manuscript submitted 24 March 2003 to Scientific Editor's Office.

Manuscript approved for publication 5 March 2004 by the Scientific Editor.

Fish. Bull. 102:464–472 (2004).

Marine sanctuaries, also known as marine protected areas (MPAs), marine reserves, and no-take areas, are being widely promoted and implemented. Important for assessing the impact of these "no-take" sanctuaries (from which fishing has been excluded) on exploited populations is the rate of emigration of animals out into the remaining fished habitat.

The most widely available data for estimating movement rates of commercially or recreationally exploited populations are those from tagged and recovered fish (Hilborn, 1990). Animals are captured alive, a visible numbered tag is inserted and they are released back into the wild. Because the accuracy of tag-recovery studies relies on fishermen reporting recaptured tags, the quality of tag-recovery information is lower than that from a controlled experiment.

Tag-recovery experiments have three limitations for estimating movement rates of animals—the first two apply to most tagged populations, the third applies specifically to emigration from sanctuaries: 1) times at large (the numbers of days from when each animal is tagged and released to when it is subsequently recaptured in the fishery) are highly variable; 2) not all recaptured tags are reported to researchers by fishermen and this rate of tag nonreporting is often unknown; and 3) tag recoveries cannot be obtained from within sanctuaries for the simple reason that no fishing is allowed there.

If this last asymmetry (of recaptures from the sanctuary coming only from tagged animals that emigrate) is not accounted for in the estimation model, then the emigration rate out of the sanctuary will be overestimated. With previous movement estimators, tag releases and recaptures from all strata have been assumed. The aim of the present article is to develop an unbiased estimator of emigration rate from no-take areas by using data of tag releases both into the sanctuary and into the fished zone surrounding it, but where recoveries from nonmoving tagged animals are only possible from the fished zone. An estimate for the recovery rate (proportion of fish recaptured and their tags reported) in the fished zone was also obtained.

Materials and methods

Tag-recovery data

The data used to estimate the emigration rate from Gleesons Landing Lobster Sanctuary (Fig. 1) are tag recoveries from lobsters tagged and released both inside the sanctuary and into the fished zone surrounding the sanctuary. A large South Australian lobster tagging program was undertaken in 1993–96 throughout South Australian waters. T-bar tags (Hallprint, Victor Harbour, South Australia) were inserted into the ventral muscle at the first segment of the lobster abdomen. The rate of tag shedding was estimated from double tags at between 6% and 12% per year (Xiao[1]) and is incorporated in the recovery rate.

[1] Xiao Y. 2003. Personal commun. Aquatic Sciences, South Australian Research and Development Institute (SARDI), P.O. Box 120, Henley Beach, South Australia 5022, Australia.

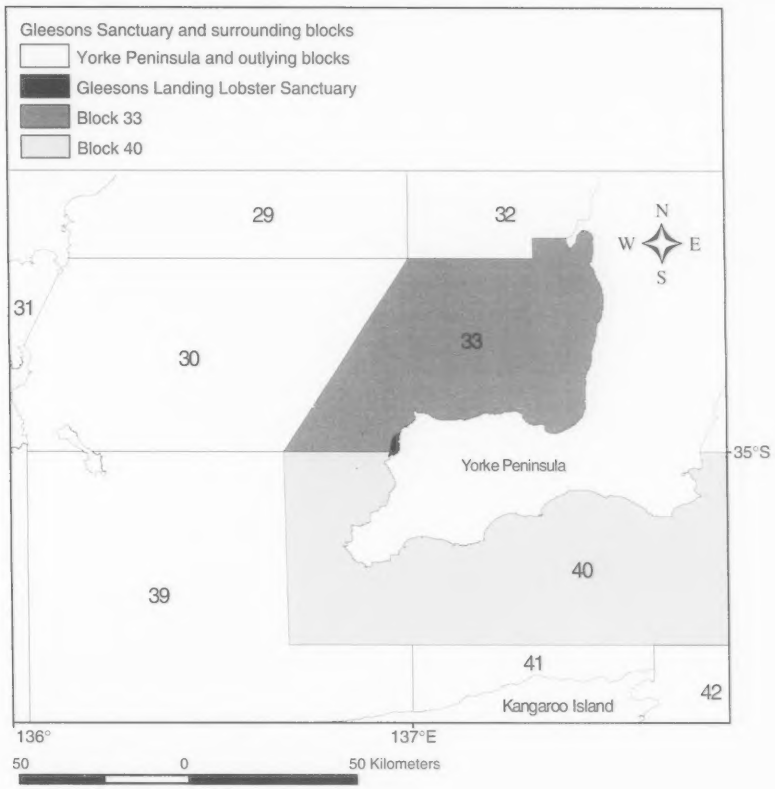

Figure 1

Location of Gleesons Landing Lobster Sanctuary (small dark area on the boundary of MFA blocks 33 and 40) along the west coast of the Yorke Peninsula in South Australia.

As part of this tagging program (Table 1), 3235 southern rock lobsters (*Jasus edwardsii*) were tagged and released into the "fished zone" surrounding Gleesons Sanctuary, namely into statistical reporting blocks 33 and 40 (Fig. 1). In January 1994, 413 lobsters were captured, tagged, and released inside the Gleesons Sanctuary. These lobsters were predominantly in the range of 80–120 mm carapace length (CL), around the size of maturity of about 100 mm CL; more lobsters below the legal minimum length (98.5 mm CL) were released in the fished zone.

Gleesons Landing Lobster Sanctuary (Fig. 1) is an area where lobster fishing has been prohibited since 1982. It lies along the Yorke Peninsula's western coast in an area of medium to low lobster catches. In width, this sanctuary extends 1–2 km from shore to seaward and runs 7–8 km north-south.

Nearly all tag recoveries were reported by commercial lobster fishermen who noticed tagged lobsters in their catch in the course of day-to-day fishing operations. Tag recoveries of lobsters released into both the sanctuary and the fished zone were, therefore, only possible from the fished zone. GPS coordinates, date, and carapace length were recorded for all tagged and recaptured lobsters. Longer-range movements from both sanctuary and fished zone were directed southwest towards the shelf edge.

Prescott et al.[2] previously described qualitative features of the movement of South Australian *Jasus edwardsii*: 1) nearly all longer-distance movements were directed offshore to deeper water and away from the coast; 2) in order of greater to lesser average distances moved, were i) immature females, ii) males, and iii) mature or egg-bearing females, for nearly all five South Australian regions analyzed; 3) movements were largely restricted to lobsters in a specific length range at time

[2] Prescott, J., R. McGarvey, G. Ferguson, and M. Lorkin. 1998. Population dynamics of the southern rock in South Australian waters. Fisheries Research and Development Corporation of Australia Report 93/086, p. 23–27. Aquatic Sciences, South Australian Research and Development Institute (SARDI), P.O. Box 120, Henley Beach, South Australia 5022, Australia.

Table 1

Tag-recovery data from Gleesons Landing lobster sanctuary and the surrounding fishing zone used in estimating yearly movement rates of southern rock lobsters.

Data	Variable name	Observed number of lobsters
Number of lobsters tagged and released into the sanctuary	$\tilde{N}_T^S$	413
Number of lobsters recovered that had moved (≥3 km) from the sanctuary into the fishing zone	$\tilde{N}_{M,R}^S$	29
Number of lobsters tagged and released into the surrounding fishing zone	$\tilde{N}_T^F$	3235
Number of lobsters recovered that had moved (≥3 km) within the fishing zone	$\tilde{N}_{M,R}^F$	89
Number of lobsters ecovered that had not moved (≥3 km) within the fishing zone	$\tilde{N}_{NM,R}^F$	277

of tagging, roughly 100–140 mm CL for females, and 100–150 mm CL for males, with a noticeable shift to smaller sizes for both sexes on the southeast coast of South Australia where growth and thus size of maturity are known to be lower; 4) overall, most lobsters in the fished areas did not move large distances, about 15% moving more than 5 km; 5) two areas stood out as being habitats from where significant movement occurred, the coastal zone off the Coorong and Yorke Peninsula; and 6) for Yorke Peninsula, higher than proportional numbers of tagged lobsters that moved significant distances were tagged and released inside Gleesons Sanctuary.

In the present study study, a lobster was classified as having undergone movement if its measured distance from point of tagging to point of recapture was greater than 3 km. This definition of lobster "movement" was chosen for two reasons. 1) The mean width of MPA coastal zone to be protected in the currently proposed state representative system is assumed to be 5 km wide; that is, it is assumed that sanctuary areas will extend from the shore outward to sea across the full 3 nmi (which is about 5 km) of state territorial waters. Thus, a 3-km movement would represent slightly more than the mean distance needed for lobsters to leave the state-protected territorial waters of the reserve and enter waters open for fishing. This assumption is strengthened by the knowledge that most longer-range movements of South Australian rock lobster are directed from inshore to offshore. 2) According to the geographical features of the present study, a 3-km movement seaward from any location in Gleesons Landing Sanctuary would place the tagged lobster well into the fished zone, i.e., it would constitute a movement out of the sanctuary. Of sanctuary-tagged lobsters, 4 of 33 recaptured lobsters in the first season after tagging exited the reserve but moved less than 3 km. These 4 recaptured lobsters were excluded from the data set. The mean distance moved by lobsters from the sanctuary was 37.4 km.

Because movement of South Australian lobsters is directed strongly away from the inshore zone, the immigration rate of lobsters back into the Gleesons Landing Sanctuary is likely to be quite low. Moreover, *Jasus edwardsii* seek shelter daily and remain on specific reefs through most of their life (MacDiarmid et al. 1991; Kelly 2001). Long-distance movements occur rarely more than once in a lifetime. Thus, in the fishing zone, where there is a continual removal of adult lobsters from reef habitat, the on-going creation of new shelter space is higher than in the sanctuary and thus lobsters that did stray inshore into the sanctuary would be less likely to find shelter, further reducing the probability of migration into the sanctuary. In the estimator presented below, only the emigration rate (the movement rate out of the sanctuary) is calculated.

The recapture data included lobsters at large for a wide range of times, many having been recaptured longer than one year after tag release. However, to estimate emigration rate, we sought the proportion of lobsters emigrating out per year. Therefore, subsets of recapture data were selected that had a mean time at large of one year. The temporal distributions of recaptured lobsters showed distinct modes around 1 year at large (recaptures between 0.5 and 1.5 years at large, Fig. 2), and the number of recaptures in these 1-year modes were used for estimating yearly movement rate (Table 1).

Some tagged and released lobsters were recaptured more than once. For these lobsters, the single recapture was selected and used for which the time at large was closest to one full year.

Notation

The information on movement in each set of tag releases is taken to be binary: each recaptured animal is classified as having moved or as having not moved during its approximately 1-year time at large (from time of tag release to time of recapture).

To carry out the movement-rate estimation, it is useful to consider the complete set of four possible outcomes for each tagged and released animal: 1) it moved and was recovered after one year (denoted M,R); 2) it did not move and was recovered after one year (NM,R); 3) it moved and was not recovered after one year (M,NR); 4) it did not move and was not recovered after one year (NM,NR). These four possible recapture outcomes applied to animals tagged and released in both strata,

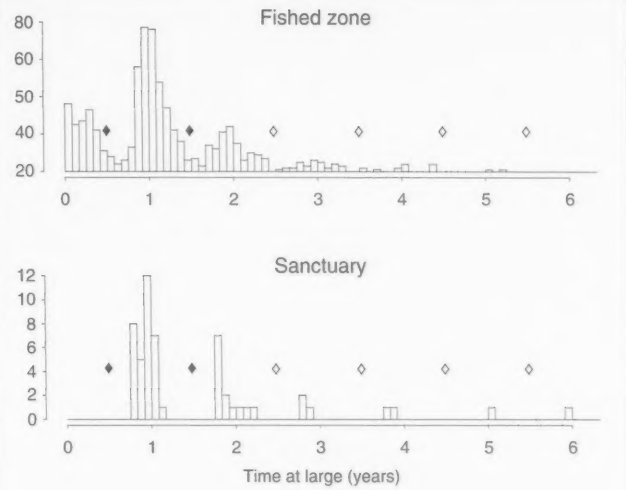

Figure 2

Histograms over time at large (in monthly bins) of recapture numbers from the fishing zone (MFA blocks 33 and 40) and from the Gleesons Landing Lobster Sanctuary. The diamond markers indicate divisions between modes at 0.5, 1.5., 2.5, etc. years at large; recaptures from the sanctuary and fishing zone that occurred between 0.5 and 1.5 years after release (between the black diamond markers) identify the subsets of data used to estimate yearly emigration rate from the sanctuary.

inside and outside the sanctuary. The tag-recovery data provided direct measures for only three of these eight possible numbers of recaptures.

We define "not recovered" to include both tagged animals that were not recaptured, as well as those that were recaptured by a fisherman but whose tag information (notably the location of recapture) was not reported back to researchers and therefore was not included in the tag-recovery database.

The movement-rate estimate is given in terms of the following data inputs: the number of lobsters tagged and released in 1) fished and 2) protected zones, and the numbers recovered that 3) moved (≥3 km) or 4) did not move from the fished zone over one year after tagging, and the 5) number that moved (≥3 km) from the sanctuary in one year.

Superscripts 'F' and 'S' denote fished zone and sanctuary, respectively, for the location of tag release. Let $N^F_{NM,R}$ and $N^F_{M,R}$ denote the numbers of animals that were recovered after a year and that moved or that did not move in the fished zone. From animals tagged and released inside the sanctuary, only the number that moved and were recovered ($N^S_{M,R}$) is available as an unbiased measure. In addition, we know the total number of animals originally tagged and released in the fished zone and sanctuary, N^F_T and N^S_T. Input quantities from the tag-recovery data set will henceforth be indicated by a tilde (~): $\{\tilde{N}^S_{M,R}, \tilde{N}^S_T, \tilde{N}^F_{NM,R}, \tilde{N}^F_{M,R}, \tilde{N}^F_T\}$ (Table 1).

Assumptions

Three assumptions were used to derive an emigration-rate estimate: 1) The two ways to define an estimate for the proportion that moved within the fished zone, namely as a proportion by using only recapture numbers, and as a proportion over the number originally tagged, can be set equal. 2) Recapture probabilities of animals that were tagged and released inside the sanctuary and that moved are assumed to equal those that were tagged and released into the fished zone and that also moved. (The first two assumptions were employed explicitly in steps 2 and 3 below.) 3) A third assumption is implicit in step 2, specifically in the recapture-conditioned movement proportion in the fished zone ($P^{F,R}_M$, Eq. 2): recapture probabilities of animals tagged and released in the fished zone that moved and of those that did not move are assumed to be equal. Assumptions 2 and 3 would both follow from assuming equal recapture probabilities for all lobsters in the fished zone.

Emigration rate: derivation of the estimate formula

In this section, an emigration-rate formula is derived. It provides a closed-form estimate of the yearly proportion of lobsters emigrating out of the sanctuary.

The proportion of animals moving can be estimated from tag-recovery data in two ways, namely as "tag-

conditioned" and "recapture-conditioned" proportions. A tag-conditioned movement proportion (Eq. 1) is the total number of lobsters that moved (≥3 km) divided by the number originally tagged and released. It includes, in the numerator, all tagged animals that moved, both those that were recovered, as well as those that were not recovered. With a recapture-conditioned movement-rate estimate (e.g., Eq. 2), only counts of recaptured lobsters are used. The estimate expresses the movement proportion as the number of tagged animals that were recaptured and that also moved (≥3 km) divided by the total number recaptured. These two definitions for the movement proportion will be used to derive an estimation formula in terms of the five data inputs.

Step 1 The derivation begins by writing the estimate for proportion of lobsters that moved (P_M^S) in tag-conditioned form:

$$P_M^S = \frac{\tilde{N}_{M,R}^S + N_{M,NR}^S}{\tilde{N}_T^S}. \quad (1)$$

This estimate of movement rate from the sanctuary is based on a tag-conditioned proportion because we have no observations of recaptured lobsters from the sanctuary that did not move (no unbiased measure of $N_{NM,R}^S$) which a recapture-conditioned movement proportion would have required. However we did have information about $N_{M,NR}^S$, the nonrecovery of tagged animals that emigrate from the sanctuary into the fished zone. It can be estimated (steps 2 and 3) with the second assumption that recovery rate for lobsters moving from the sanctuary equals that of lobsters moving (≥3 km) within the fished zone.

Step 2 Under assumption 1, the two ways in which movement proportion in the fished zone can be defined (as tag- and recapture-conditioned proportions) are equated. For fished zone releases, the recapture-conditioned ('*rc*') movement proportion is written

$$P_M^{F,rc} = \frac{\tilde{N}_{M,R}^F}{\tilde{N}_{NM,R}^F + \tilde{N}_{M,R}^F}. \quad (2)$$

For the recapture-conditioned estimate formula (Eq. 2), all three quantities on the right-hand side are given as data inputs. With only numbers of lobsters recovered, the formula is, in this sense, conditional on recapture.

The tag-conditioned ('*tc*') proportion of lobsters moving ≥3 km of those released in the fished zone is written

$$P_M^{F,tc} = \frac{\tilde{N}_{M,R}^F + N_{M,NR}^F}{\tilde{N}_T^F}. \quad (3)$$

The first assumption is

$$P_M^{F,rc} = P_M^{F,tc}. \quad (4)$$

Substituting Equations 2 and 3 into Equation 4 and solving for $N_{M,NR}^F$, the number of lobsters that moved ≥3 km within the fished zone but were not recovered, yields

$$N_{M,NR}^F = \tilde{N}_{M,R}^F \left\{ \frac{\tilde{N}_T^F}{\tilde{N}_{NM,R}^F + \tilde{N}_{M,R}^F} - 1 \right\}. \quad (5)$$

Step 3 Assumption 2 permits the derivation of a formula for $N_{M,NR}^S$. We first define the recovery proportions of animals that moved within the fished zone (F) as

$$f_M^F = \frac{\tilde{N}_{M,R}^F}{\tilde{N}_{M,NR}^F + \tilde{N}_{M,R}^F} \quad (6)$$

and from the sanctuary (S) as

$$f_M^S = \frac{\tilde{N}_{M,R}^S}{N_{M,NR}^S + \tilde{N}_{M,R}^S}. \quad (7)$$

Assumption 2, that the recovery rate (necessarily in the fished zone) for animals that were tagged and released in the sanctuary and that moved into the fished zone is the same as for animals that were both released and recaptured after moving within the fished zone becomes

$$f_M^F = f_M^S. \quad (8)$$

Substituting Equations 6 and 7 into Equation 8 and rearranging terms, we have

$$N_{M,NR}^S = \frac{\tilde{N}_{M,R}^S \left(N_{M,NR}^F + \tilde{N}_{M,R}^F \right)}{\tilde{N}_{M,R}^F} - \tilde{N}_{M,R}^S. \quad (9)$$

Step 4 Substituting Equation 5 into Equation 9 and substituting the result into Equation 1 yields a closed-form estimation formula for the quantity we seek, the proportion moving from the sanctuary in one year:

$$P_M^S = \frac{\tilde{N}_T^F \cdot \tilde{N}_{M,R}^S}{\tilde{N}_T^S \cdot (\tilde{N}_{NM,R}^F + \tilde{N}_{M,R}^F)}. \quad (10)$$

Numerical estimator: double-hypergeometric likelihood method

A likelihood formulation of this estimator was also constructed. The likelihood function describing a single tag-recapture experiment is hypergeometric (Seber, 1982; Rice, 1995) because sampling is without replacement. The set of possible outcomes from each of the two tagging experiments can be formulated as a 2×2 contingency table for the experimental populations of all lobsters originally tagged and released. The two pairs of outcomes represented in each contingency table are "moved" or "not moved" and "recovered" or "not recovered," yielding the four possible outcomes from both sets of tag releases (see "Notation" section).

In this study the data from two interacting tag-recovery experiments were used to generate an estimate of reserve emigration rate, namely of lobsters tagged and released into the sanctuary and into the fished zone. Thus, the product of a pair of linked hypergeometric probability mass functions, each corresponding to a 2-way contingency table, is the natural form of the likelihood function for P_M^S.

The derivation of Equation 10 was made with two assumptions, namely Equations 4 and 8. Incorporated in the likelihood, the two assumptions constrain the eight recapture numbers in the contingency tables. In the likelihood formulation, a third constraint was needed which is analogous to assumption 1 but which applies to sanctuary releases.

The derivation for constructing this likelihood from a pair of linked hypergeometric probability functions will proceed by 1) writing out the "raw" contingency tables in terms of the eight recapture numbers (N), as denoted in the "Tag-recovery data" and "Notation" sections, 2) algebraically re-expressing the elements of the tables so that the parameter to be estimated is explicit, 3) imposing the three constraints, and 4) writing out the likelihood, using the hypergeometric form for contingency tables.

For the lobsters tagged and released in the sanctuary, the raw contingency table is

	Recovered	Not recovered	Totals
Moved	$\tilde{N}^S_{M,R}$	$N^S_{M,NR}$	$\tilde{N}^S_{M,R}+N^S_{M,NR}$
Not moved	$N^S_{NM,R}$	$N^S_{NM,NR}$	$\tilde{N}^S_T-(\tilde{N}^S_{M,R}+N^S_{M,NR})$
Totals	$\tilde{N}^S_{M,R}+N^S_{NM,R}$	$\tilde{N}^S_T-(\tilde{N}^S_{M,R}+N^S_{NM,R})$	$\tilde{N}^S_T$

For the lobsters tagged in the fished zone:

	Recovered	Not recovered	Totals
Moved	$\tilde{N}^F_{M,R}$	$N^F_{M,NR}$	$\tilde{N}^F_{M,R}+N^F_{M,NR}$
Not moved	$\tilde{N}^F_{NM,R}$	$N^F_{NM,NR}$	$\tilde{N}^F_T-(\tilde{N}^F_{M,R}+N^F_{M,NR})$
Totals	$\tilde{N}^F_{M,R}+\tilde{N}^F_{NM,R}$	$\tilde{N}^F_T-(\tilde{N}^F_{M,R}+\tilde{N}^F_{NM,R})$	$\tilde{N}^F_T$

The two hypergeometric probability mass functions (pmfs) giving the model-predicted proportion of lobsters that moved and were recovered, based on the two contingency tables, are written as

$$P(\tilde{N}^S_{M,R})=\left(\frac{\binom{\tilde{N}^S_{M,R}+N^S_{M,NR}}{\tilde{N}^S_{M,R}}\binom{\tilde{N}^S_T-(\tilde{N}^S_{M,R}+N^S_{M,NR})}{N^S_{NM,R}}}{\binom{\tilde{N}^S_T}{\tilde{N}^S_{M,R}+N^S_{NM,R}}}\right) \quad (11)$$

$$P(\tilde{N}^F_{M,R})=\left(\frac{\binom{\tilde{N}^F_{M,R}+N^F_{M,NR}}{\tilde{N}^F_{M,R}}\binom{\tilde{N}^F_T-(\tilde{N}^F_{M,R}+N^F_{M,NR})}{\tilde{N}^F_{NM,R}}}{\binom{\tilde{N}^F_T}{\tilde{N}^F_{M,R}+\tilde{N}^F_{NM,R}}}\right). \quad (12)$$

Because the goal is to estimate the movement proportion, P_M^S (rather than any specific value of N), this proportion will need to be made explicit in the likelihood function as the sole freely varying parameter. Substituting from the definition of P_M^S (Eq. 1), we have

$$N^S_{M,NR}=P^S_M\cdot\tilde{N}^S_T-\tilde{N}^S_{M,R}. \quad (13)$$

Substituting for all occurrences of $N^S_{M,NR}$, Equation 11 becomes

$$P(\tilde{N}^S_{M,R})=\left(\frac{\binom{P^S_M\cdot\tilde{N}^S_T}{\tilde{N}^S_{M,R}}\binom{\tilde{N}^S_T\cdot(1-P^S_M)}{N^S_{NM,R}}}{\binom{\tilde{N}^S_T}{\tilde{N}^S_{M,R}+N^S_{NM,R}}}\right). \quad (14)$$

Writing the full joint-likelihood expression formed by the product of the two hypergeometric pmfs gives

$$L=\left(\frac{\binom{\tilde{N}^F_{M,R}+N^F_{M,NR}}{\tilde{N}^F_{M,R}}\binom{\tilde{N}^F_T-(\tilde{N}^F_{M,R}+N^F_{M,NR})}{\tilde{N}^F_{NM,R}}}{\binom{\tilde{N}^F_T}{\tilde{N}^F_{M,R}+\tilde{N}^F_{NM,R}}}\right)\times\left(\frac{\binom{P^S_M\cdot\tilde{N}^S_T}{\tilde{N}^S_{M,R}}\binom{\tilde{N}^S_T\cdot(1-P^S_M)}{N^S_{NM,R}}}{\binom{\tilde{N}^S_T}{\tilde{N}^S_{M,R}+N^S_{NM,R}}}\right).$$

As formulated, the value of $N^S_{NM,R}$ is still undetermined by data or constraint. A third constraint is therefore required. As with assumption 1 for the fished zone (Eq. 4), we apply the assumed equivalence of tag- and recapture-conditioned proportions to the sanctuary releases:

$$P^{S,rc}_M=\tilde{N}^S_{M,R}/(N^S_{NM,R}+\tilde{N}^S_{M,R})=P^{S,tc}_M=(\tilde{N}^S_{M,R}+N^S_{M,NR})/\tilde{N}^S_T.$$

In this application, $N^S_{NM,R}$ is understood as the number of lobsters that would have been taken if fishing had not been excluded from the sanctuary. Solving for $N^S_{NM,R}$ yields the third constraint,

$$N^S_{NM,R}=(\tilde{N}^S_{M,R}\cdot\tilde{N}^S_T)/(\tilde{N}^S_{M,R}+N^S_{M,NR})-\tilde{N}^S_{M,R},$$

Table 2

Intermediate calculated quantities from the numerical estimation. The equalities of $P_M^{F,rc}=P_M^{F,tc}$ and $f_M^F=f_M^S$ state assumptions 1 and 2.

Intermediate quantity	Variable name	Estimate
The proportions of lobsters tagged in the fishing zone that moved (≥3 km); recapture-conditioned ($P_M^{F,rc}$) or tag-conditioned ($P_M^{F,tc}$)	$P_M^{F,rc}=P_M^{F,tc}$	0.243
Number of lobsters that moved but were not recovered in the fishing zone	$N_{M,NR}^F$	697.7
Number of lobsters that moved from the sanctuary but were not recovered	$N_{M,NR}^S$	227.3
Number of lobsters that did not move and would have been recovered had there been equivalent levels of harvesting in the sanctuary	$N_{NM,R}^S$	17.7
Recovery proportions (in the fishing zone)—assumed to be equal for lobsters that moved inside the fishing zone f_M^F or from the sanctuary	$f_M^F=f_M^S$	0.113

without which this numerical estimator did not converge.

The factorial terms in the binomial coefficients of Equations 12 and 14 are defined only for natural numbers. However, in numerical minimization, factorials must be replaced with continuously varying approximations because the negative log-likelihood objective function is minimized by using numerical derivatives. The factorial z! was extended from natural numbers to the real line by using the gamma function, $\Gamma(z+1)$ and by using an asymptotic approximation formula for $\ln \Gamma(z)$ (Eq. 6.1.41 in Abramowitz and Stegun, 1965):

$$\ln\Gamma(z)=\left(z-\frac{1}{2}\right)\ln(z)-z+\frac{1}{2}\ln(2\pi)+\frac{1}{12z}-\frac{1}{360z^3}+\frac{1}{1260z^5}-\frac{1}{1680z^7}+\frac{1}{1188z^9}-\frac{691}{360360z^{11}}+\frac{1}{156z^{13}}. \quad (15)$$

The negative log likelihood was minimized numerically by using the AD Model Builder parameter estimation software (http://otter-rsch.com/admodel.htm).

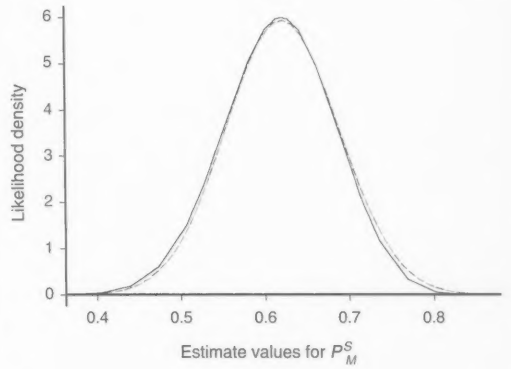

Figure 3

Profile likelihood (solid line) and asymptotic normal approximation (dashed line) for the likelihood confidence range about the estimate of P_M^S.

Results

The closed-form estimator for the proportion of lobsters that moved from the sanctuary (P_M^S) gave an estimate of 0.6206; i.e., about 62% of the lobsters tagged in Gleesons Sanctuary moved out in one year. The estimate obtained numerically, by maximizing the double-hypergeometric likelihood, yielded a value of 0.6212.

The small difference between the analytic and numerical estimates (0.09%) is presumably due to the use of the numerical approximation for the log-gamma function by the expansion of Equation 15. The close agreement suggests that the error introduced by that approximation is small.

The AD Model Builder parameter estimation software allows one to estimate confidence intervals of the movement-rate estimate in two ways: asymptotically, as diagonal elements of the covariance matrix, and by using a profile likelihood. Confidence intervals for the emigration rate estimate were thus obtained numerically from the hypergeometric likelihood by using both the asymptotic normal approximation and an exact profile likelihood. These gave 95% errors of 21.2% and 21.5% of the estimate, respectively. The approximate normal probability density function and the profile likelihood probability density function were also plotted (Fig. 3), yielding close agreement. Asymptotic confidence intervals therefore appear satisfactory for emigration proportion estimates not lying near the bounds of 0 and 1.

Intermediate calculation results (Table 2) included the recovery rate and movement rate (≥3 km) within the fished zone.

When independent estimates of exploitation rate are available, typically from stock assessment, the rate of tag reporting can be calculated from the tag-estimated recovery rate. The exploitation rate (yearly proportion of legal-size lobsters harvested) for the recapture year and

location of the present study (the 1995 northern zone rock lobster season) was estimated to be 26% (Ward et al.[3]) by using total yearly effort and catches by weight and number and a vector of weights at age. The tag-recovery rate of 11.3% (Table 2) is the estimated proportion of tagged lobsters that were captured and for which tags were reported. Thus the estimated tag-reporting rate (of those recaptured) is 0.113/0.26=43%. If tag shedding and natural mortality were also incorporated as additional causes for nonrecovery, the estimate would fall in the neighborhood of a 50% tag-reporting rate. This estimated level of tag-reporting falls within the range considered probable by fishermen. Thus, the recovery-rate estimate falls within a plausible range of values, adding confidence that the tag-recovery data are consistent with external estimates of exploitation rate.

Substantial movement of *Jasus edwardsii* out of a marine sanctuary was previously observed in New Zealand (Kelly and MacDiarmid, 2003) but not in Tasmania (Gardner and Ziegler[4]). Long-distance movement of this genus was also observed in New Zealand (Booth, 1997) but was much less common in Tasmanian *Jasus edwardsii* populations (Gardner et al., in press).

Discussion

The emigration-rate derivation above combined recapture-and tag-conditioned movement proportions. Both ways to define a movement rate were used to constrain the range of solutions for both analytic and numerical estimators. Equating these two definitions for movement proportion reduced the degrees of freedom by 1, thereby circumventing the absence of a count of recaptured lobsters from within the fished zone.

Previous estimators of movement rates among spatial cells from tag-recovery data have used either tag- or recapture-conditioned approaches. Hilborn (1990; see also Quinn and Deriso, 1999) developed a tag-conditioned movement-rate estimator. This estimator generally requires prior knowledge of the tag reporting rate. Schwarz et al. (1993) employed data consisting of simultaneous tag releases and recaptures repeated over a number of years at the same time each year to estimate movement, survival, and recovery rates in each spatial stratum. Schwarz et al. (1993) presented a general formulation for modeling this multiple yearly tag-recovery data set, extending a series of estimators for movement and survival (Arnason, 1972, 1973), and estimated the rate of tag recovery. Brownie et al. (1993) generalized the estimator of Schwarz et al. to non-Markovian movement rates. McGarvey and Feenstra (2002), following Hilborn, used the less costly and more commonly available single tag-recovery data employed in the present study but adopted a recapture-conditioned approach for estimating yearly movement rates. With "numbers recaptured" appearing in both the numerator and denominator, all nonspatially dependent sources of variation (such as tag reporting and shedding, short- and long-term tag-induced mortality, and natural mortality) cancel from the predicted recapture-conditioned likelihood proportions. This procedure permits a corresponding reduction in the prior information required to obtain unbiased movement estimates.

When recapture times vary, movement estimation is sensitive to spatial differences in mortality rate, notably between tag and recapture cells. Assuming that the nonreporting rate is unknown, mortality can be inferred from single tag-release information only imprecisely, for example by using mean tagged time at large. For this reason externally obtained mortality estimates, typically from stock-assessment models using fishery data, can be usefully combined with single tag recoveries in movement estimation. Hestbeck (1995) showed, when survival differs by cell, that ignoring the time of movement between yearly samples could bias movement estimates. McGarvey and Feenstra (2002) made explicit the variation in residence time and thus survival in source (tag-release) and destination (recapture) cells for each recaptured animal. By using prior knowledge of a migration season, migration source cell and destination cell residence times can be approximated as the time from the date of tag release to an assumed fixed (yearly) date of movement, and from that date to the date of recapture. These residence times are used in exponential survival factors that differ spatially given externally estimated fishing mortality rates in each cell.

For the data set available from Gleesons Landing, all tagged animals were released during the peak fishing season (mid-summer). Thus recoveries from the following fishing season had a mean and mode near the desired one-year-at-large. In future tag-recovery studies, where a yearly movement rate is sought, a similar choice for timing of tag releases, namely during the season of highest fishery catches, should yield a peak in recaptures a year later. Schwarz et al. (1993) employed this strategy with their multiple yearly tag-recovery data sets.

In the estimator presented above, variations in expected recovery numbers versus time, notably due to survival, were neglected. The small sample (33 recoveries between 0.5 and 1.5 years from the sanctuary) and lack of recaptures from within the sanctuary necessitated more modest estimation goals. Among data classes available for movement analysis, notably 1) multiple yearly tag recaptures by researchers in all cells, 2) multiple yearly tag recoveries where recapture is by fishermen (or

[3] Ward, T. M., R. McGarvey, Y. Xiao, and D. J. Brock. 2002. Northern zone rock lobster (*Jasus edwardsii*) fishery. South Australian Fisheries Assessment Series Report 2002/04b, 109 p. Aquatic Sciences, South Australian Research and Development Institute (SARDI); P.O. Box 120, Henley Beach, South Australia 5022, Australia.

[4] Gardner, C., and P. Ziegler. 2001. Are catches of the southern rock lobster *Jasus edwardsii* a true reflection of their abundance underwater? Tasmanian Aquaculture and Fisheries Institute Final Report. TAFI (Tasmanian Aquaculture and Fisheries Institute), University of Tasmania, Private Bag 49, Hobart TAS 7001, Australia.

hunters) in all cells, 3) single tag recoveries by fishermen in all cells, and 4) the data set employed in the present study of single-tag recoveries by fishermen in one of two cells, the latter represents the low end in quality and quantity of information about movement and survival.

A time-dependent approach could theoretically extend the approach of McGarvey and Feenstra (2002) to make explicit the residence times of each recaptured individual in the fishing zone and sanctuary, respectively, and thus make explicit differences in the predicted survival rate before and after movement. However without prior knowledge of when movement took place for each recaptured lobster, a modified likelihood method is called for, requiring integration over the probable movement times between tag release and recapture. This extension of residence-time-dependent movement estimators to variable times of movement remains a topic for future research.

Acknowledgments

I thank Hugh Possingham, André Punt, and two reviewers for comments on the draft manuscript. Lobsters were tagged and released into Gleesons Landing sanctuary by Greg Ferguson, together with fishermen Lenny and Murray Williams, under Fisheries Research and Development Corporation Project 93/086. This work was supported by the Australian Fisheries Research and Development Corporation Project No. 2000/195, and by the South Australian rock lobster industry.

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Reproductive dynamics of female spotted seatrout (*Cynoscion nebulosus*) in South Carolina*

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Abstract—We describe reproductive dynamics of female spotted seatrout (*Cynoscion nebulosus*) in South Carolina (SC). Batch fecundity (BF), spawning frequency (SF), relative fecundity (RF), and annual fecundity (AF) for age classes 1–3 were estimated during the spawning seasons of 1998, 1999, and 2000. Based on histological evidence, spawning of spotted seatrout in SC was determined to take place from late April through early September. Size at first maturity was 248 mm total length (TL); 50% and 100% maturity occurred at 268 mm and 301 mm TL, respectively. Batch fecundity estimates from counts of oocytes in final maturation varied significantly among year classes. One-year-old spotted seatrout spawned an average of 145,452 oocytes per batch, whereas fish aged 2 and 3 had a mean BF of 291,123 and 529,976 oocytes, respectively. We determined monthly SF from the inverse of the proportion of ovaries with postovulatory follicles (POF) less than 24 hours old among mature and developing females. Overall, spotted seatrout spawned every 4.4 days, an average of 28 times during the season. A chronology of POF atresia for water temperature >25°C is presented. Length, weight (ovary-free), and age explained 67%, 65%, and 58% of the variability in BF, respectively. Neither RF (number of oocytes/g ovary-free weight) nor oocyte diameter varied significantly with age. However, RF was significantly greater and oocyte diameter was smaller at the end of the spawning season. Annual fecundity estimates were approximately 3.2, 9.5, and 17.6 million oocytes for each age class, respectively. Spotted seatrout ages 1–3 contributed an average of 29%, 39%, and 21% to the overall reproductive effort according to the relative abundance of each age class. Ages 4 and 5 contributed 7% and 4%, respectively, according to predicted AF values.

Manuscript submitted 13 May 2002 to Scientific Editor's Office.

Manuscript approved for publication 19 March 2004 by the Scientific Editor.

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The spotted seatrout (*Cynoscion nebulosus*) is an estuarine-dependent member of the family Sciaenidae. Spotted seatrout are year-round residents of estuaries along the South Atlantic coast and spawning takes place inshore and in coastal areas (McMichael and Peters, 1989; Mercer[1]; Luczkovich et. al.[2]). As in many other sciaenids, spawning in this species occurs in the evening (Holt et al., 1985). Male spotted seatrout have the capacity to produce "drumming" sounds that are caused by the contraction of the swimbladder by specialized muscles that are seasonally hypertrophied from the abdominal hypaxialis muscle mass (Fish and Mowbray, 1970; Mok and Gilmore, 1983). Direct involvement of sound production with spawning has been shown for this and other sciaenids (Mok and Gilmore, 1983; Saucier et al., 1992; Saucier and Baltz, 1993; Luczkovich et al.[2]).

We have collected information on the spawning behavior of spotted seatrout in coastal South Carolina since 1990 (Saucier et al., 1992; Riekerk et al.[3]). Spawning aggregations were located by listening for drumming sounds from late afternoon until ~2300 h with passive hydrophone equipment. Spawning activity was subsequently verified through collections of newly spawned eggs and by the rearing of the larvae in the laboratory (Saucier et al., 1992).

Spotted seatrout are group-synchronous spawners with indeterminate fecundity and the protracted spawning season extends from April through September along the South Atlantic and Gulf of Mexico coasts (Overstreet, 1983; Brown-Peterson et al., 1988; McMichael and Peters, 1989; Saucier and Baltz, 1993; Brown-Peterson and Warren, 2001; Brown- Peterson et al., 2002; Nieland et al., 2002, Brown-

* Contribution 539 from the Marine Resources Research Institute of the South Carolina Department of Natural Resources, Charleston, SC 29422-2559.

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[2] Luczkovich, J. J., H. J. Daniel III and M. W. Sprague. 1999. Characterization of critical spawning habitats of weakfish, spotted seatrout and red drum in Pamlico Sound using hydrophone surveys. Final report and annual performance report F-62-2 and F-62-2, p 65–68. North Carolina Department of Environment and Natural Resources, Division of Marine Fisheries, Morehead City, NC 28557.

[3] Riekerk, G. H. M., S. J. Tyree, and W. A. Roumillat. 1997. Spawning times and locations of spotted seatrout in the Charleston Harbor Estuarine System from acoustic surveys, 21 p. Final Report to Charleston Harbor Project, Bureau of Ocean and Coastal Resources Management, South Carolina Department of Health and Environmental Control, 1362 McMillan Ave., Charleston, SC 29405.

Peterson, 2003; Wenner et al.[4]). As in other indeterminate spawning fish, annual fecundity in this species is determined by the number of oocytes released during each spawning event (batch fecundity) and the number of spawning events occurring during the course of the spawning season (spawning frequency). Early efforts to estimate fecundity for spotted seatrout did not take into account the repetitive nature of spawning activities in this species (Pearson, 1929; Sundararaj and Suttkus, 1962; Overstreet, 1983) and only recently has an effort been made to coordinate batch fecundities with spawning frequencies (Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001; Nieland et al., 2002). This procedure is intuitively necessary to estimate the reproductive output for an entire spawning season and is made even more useful for fisheries management if separated by size class or age cohort within a population (Prager et al., 1987; Goodyear, 1993; Zhao and Wenner[5]).

An important component of assessment for management involves determining the spawning potential ratio (SPR), a measure of the effect of fishing on the reproductive potential of a stock (Goodyear, 1993). This value is usually calculated as the ratio of spawning stock biomass per recruit (SSBR) in the presence of fishing mortality (F) to the SSBR when F is equal to zero (Gabriel et al., 1989; Goodyear, 1993). Spawning potential ratio is currently used as a biological reference point for definition of recruitment overfishing (i.e., Vaughan et al., 1992). The calculation of SPR can be improved, however, by introducing egg production into the model. Fecundity is a much better predictor of reproductive potential than female biomass. Moreover, SPR calculations based on egg production may be more sensitive to the size-age composition of the spawning stock. However, accurate annual fecundity estimates for use in stock assessment do not exist for this or many other species in need of fisheries management. Therefore, our goal was to obtain batch fecundity (BF), spawning frequency (SF), and annual fecundity (AF) estimates for spotted seatrout by age class.

Materials and methods

Data to address the main objectives of this study were collected from late April through early September 1998–2000 as part of a long term monitoring effort (1991–present) to assess the relative abundance of age classes of recreationally important finfish in South Carolina estuaries. The study followed a monthly stratified random sampling design in three estuarine systems. The Cape Romain system comprised two strata; Romain Harbor and northern Bulls Bay. The Charleston Harbor system contained four strata: the Wando, Cooper, and Ashley Rivers, and Charleston Harbor. The Ashepoo-Combahee-Edisto (ACE) Basin system comprised a single stratum (Fig. 1). The number of sampling sites within each stratum ranged from 23 to 30. A subset of 12–14 sites was randomly selected each month. Sampling was conducted only during the daytime ebbing tide (0700–1800 h), primarily over mud and oyster shell substrates adjacent to the *Spartina alterniflora* marsh. At each site, we deployed a trammel net (182.8 m long by 2.4 m deep; outer walls: 17.8 cm square [35.6 cm stretch]; inner wall: 3.2 cm square [6.4 cm stretch]) from a rapidly moving shallow water boat in an arc against the shoreline at depths ranging from 0.5 to 2.0 m. We disturbed the water within the site in an effort to frighten fishes into the entrapment gear. We then hauled the trammel net back into the boat and removed the catch, which was kept alive in a 70-liter oxygenated holding tank. Spotted seatrout were measured for total length (TL) and standard length (SL) and a subsample of fish from each effort (5–10 individuals for each 20-mm size interval per month) were sacrificed, placed on ice, and transported to the laboratory for aging and reproductive data.

Specimens were processed in the laboratory 2–12 hours after capture. We recorded standard life-history parameters (TL, SL, fish weight, gonad weight, sex, and maturity) for each specimen. The following equation was used to convert lengths when necessary:

$$TL = 5.689 + 1.167(SL)\ (r^2=0.998) \qquad n=1191.$$

We removed sagittal otoliths for aging and preserved sections (<2% by weight) of each ovary in neutral buffered formalin for histological processing. The latter involved standard procedures for paraffin embedding and sectioning, and standard hematoxylin and eosin-y staining (Humason, 1972). Histological sections were viewed under a Nikon Labophot compound microscope equipped with a teaching head so that two readers could interpret sections simultaneously. Maturity estimation was modified from that of Wenner et al.[4] (Table 1).

Size at first maturity was histologically derived by first evidence of cortical alveoli stage oocytes. To arrive at estimates of 50% and 100% maturity, data were subjected to PROBIT analysis.

Age determination

The left sagittae were marked with a soft lead pencil through the core and embedded in epoxide resin. A transverse section (~0.5-mm thick) was taken through the core by using a low-speed saw equipped with a pair

[4] Wenner, C. A., W. A. Roumillat, J. E. Moran Jr., M. B. Maddox, L. B. Daniel III, and J. W. Smith. 1990. Investigations on the life history and population dynamics of marine recreational fishes in South Carolina: part 1. Final Report F-37, 177 p. Marine Resources Research Institute, Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., Charleston, SC 29412.

[5] Zhao, B., and C. A. Wenner. 1995. Stock assessment and fishery management of the spotted seatrout, *Cynoscion nebulosus*, on the South Carolina coast, 90 p. Marine Resources Research Institute, Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., Charleston, SC 29412.

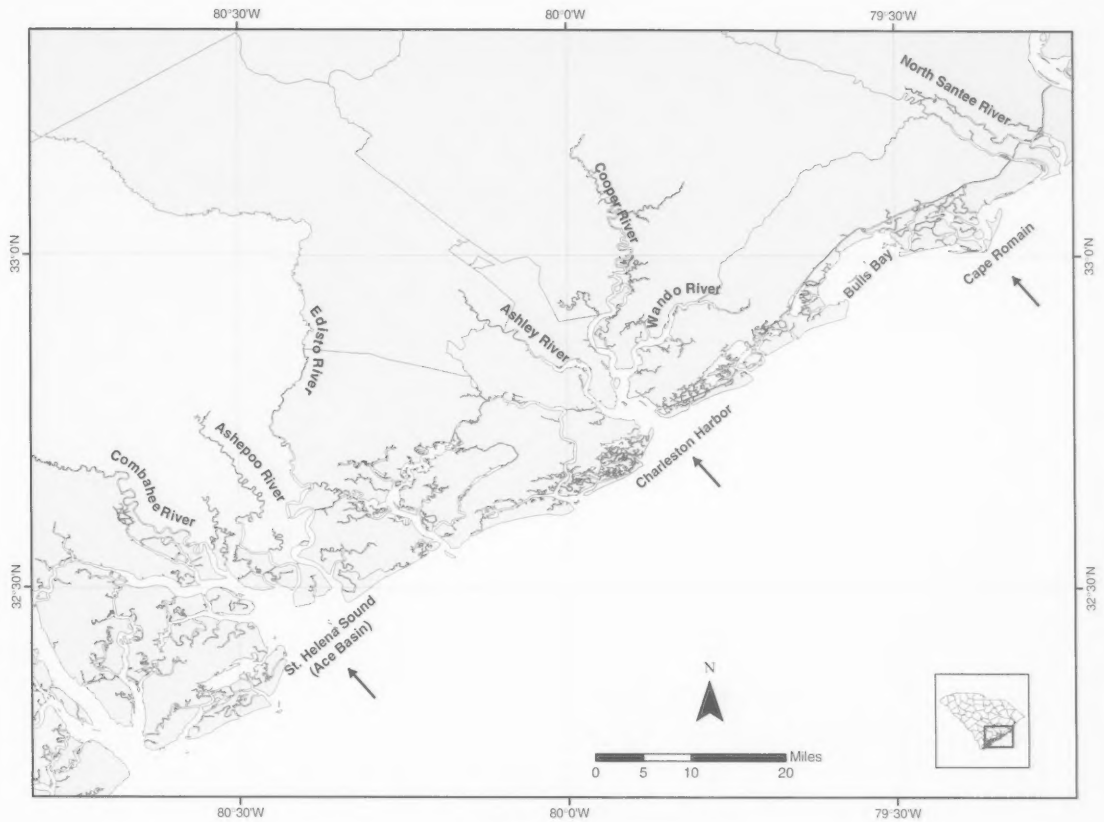

Figure 1

The three South Carolina estuarine systems (indicated by arrows) where *C. nebulosus* were collected.

Table 1

Criteria used for microscopic staging of *C. nebulosus* ovaries. FOM = final oocyte maturation; POF = postovulatory follicle.

Stage	Description
Immature	Ovary small in cross section. Early stage with only oogonia evident; later stage with small (<0.08 mm) primary oocytes, tightly packed. No evidence of early vitellogenesis.
Developing	First appearance of cortical alveoli stage oocytes through late vitellogenesis but no evidence of early FOM (lipid and yolk globule coalescence).
Ripe	Ovary containing oocytes demonstrating FOM (lipid and yolk coalescence through hydration).
Mature with day-0 POFs	Ovary exhibits POFs <24 h (see Table 2). Found at all water temperatures throughout the spawning season.
Mature with day-1 POFs	Ovary exhibits POFs >24 h consisting of closely packed granulosa cells (0.08–0.1 mm). Only identified in water < 25°C.
Spent	Ovary containing alpha- and beta-stage oocytic atresia.
Resting	Ovary containing small primary oocytes and oocytes with perinuclear nucleoli (<0.12 mm); usually some remnants of oocytic atresia.

of diamond wafering blades. The resulting section was mounted on a labeled microscope slide and examined with a Nikon SMZ-U microscope. A 1 January birth date was assumed for the process of aging. Spotted seatrout deposit an annulus in April or May (Murphy and Taylor, 1994; Wenner et al.[4]). Fish in the first three months of

Table 2

Criteria used for microscopic staging of *C. nebulosus* day-0 postovulatory follicles (POFs) in water temperatures above 25°C. Measurements represent longest axis of POFs.

POF chronology (in hours)	Description
0–4	Regular arrangement of granulosa-cell nuclei proximal to the basement membrane and obvious multiple layering as described by Hunter and Macewicz (1985). 200–300 μm (Fig. 4A).
5–8	Early signs of atresia, loss of the obvious layering, hypertrophy of granulosa cells, and a general compaction with an investment of blood vessels. 180–250 μm (Fig. 4B).
9–12	Well-defined lumen separating the internal granulosa cells from the outer wall of granulosa cells encompassed by theca. 150–200 μm (Fig. 4C).
13–24	Lumen reduced primarily by loss of granulosa tissue and proximity of peripheral layers. 130–175 μm (Fig. 4, D and E).

the year were aged by the addition of 1 year to the count of the number of annuli on the thin sections. In April or May, if the section had a large marginal increment, one was also added to the annular count. If the marginal increment was small or if the ring was detectable on the edge of the otolith section, age was equal to the number of annuli.

Seasonality

Spawning season for spotted seatrout in South Carolina was determined by using two techniques. The gonadosomatic index (GSI) was calculated as

$$(GW/OFWT) \times 100,$$

where GW = gonad weight (g); and
$OFWT$ = ovary-free weight (g).

For years prior to this study (1991–97), mean monthly GSI was obtained for all females by using data from the South Caroline Department of Natural Resources inshore fisheries archives (Wenner[6]). Reproductive seasonality among female spotted seatrout throughout the year was also examined by using histology (Table 1).

The first evidence of oocytes in final oocyte maturation (FOM) as evidenced by lipid and yolk coalescence; Brown-Peterson et al., 1988) or the occurrence of postovulatory follicles (POFs) defined the beginning of the spawning season. To determine the cessation of spawning, the percent occurrence of females in spawning condition (ripe and repeat spawners) and those in postspawning condition (spent and resting) were obtained for the months of August and September. To investigate the condition of females, we examined Fulton's condition factor (Ricker, 1975) over the spawning season using linear regression.

[6] Wenner, C. 2002. Unpubl. data. Marine Resources Research Institute, Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., Charleston, SC 29412.

Spawning frequency

We obtained samples for spawning frequency (SF) determination from 1 May through 31 August 1998, 1999 and 2000. Although samples were routinely collected throughout the year, only from early May through late August did we capture enough animals in the appropriate reproductive state for SF estimation. Spawning frequency was calculated as either the inverse of the proportion of ovaries with day-0 POFs (Hunter and Macewicz, 1985; Brown-Peterson et al., 1988) or with oocytes in FOM (Brown-Peterson et al., 1988; Lisovenko and Adrianov, 1991) among mature and developing females.

We designated two distinct morphological features of POFs based on time of specimen capture and water temperature. We interpreted the largest, least atrophied POFs to be <24 h old and termed them "day-0" POFs (Hunter and Macewicz, 1985). The presence of day-0 POFs in the ovary indicated that spawning had occurred the previous night. The second category comprised smaller POFs, which primarily consisted of closely packed granulosa cells determined to be >24 h old.

To complete the chronology of POF atresia we undertook round-the-clock sampling on 27–28 June and 26 July 2000. During these efforts, sampling continued beyond routine hours to encompass the period between dusk and dawn. The histological samples obtained allowed for the calibration of criteria used to age POFs (Table 2). To determine whether SF varied among months and age classes, Kruskal-Wallis tests were used. Because both factors (month and age) were fixed (model 1), it was not possible to test for their interaction by using a two-way parametric ANOVA without replication.

As a result of targeting fish for batch fecundity estimates (see below), we had available numerous specimens with oocytes in FOM with which to establish monthly SF. However, we knew that these specimens were disappearing from our shallow sampling sites into deeper spawning areas as the day progressed (Riekerk et al.[3]),

thus potentially adding bias to our SF estimates. Even though estimates of SF based on FOM were performed *a posteriori*, we chose to report them strictly for comparison to other studies with this method. Because sampling for females exhibiting FOM was accomplished in a directed fashion, statistical comparisons were not attempted.

Batch fecundity and relative fecundity

Observations taken over a decade of sampling the Charleston Harbor estuarine system showed that in females captured in shallow water (<1.5 m) during the spawning season, FOM began at about 1200 h (Wenner[6]). Similarly, Crabtree and Adams[7] reported FOM beginning in Florida spotted seatrout at about mid-day. Lowerre-Barbieri et al.[8] found hydrated females in shallow water in the vicinity of aggregations of drumming males in deeper water. We also speculated that from mid- to late afternoon hydrated females moved along the marsh edge toward deeper water spawning aggregations (8–25 m). Hydrophone surveys conducted in the Charleston Harbor area over several years (Riekerk et al.[3]; Wenner[6]) indicated that noise production typically began around 1800 h and ceased around 2200 h. Because this behavior has been associated with spawning in this and other sciaenids (Mok and Gilmore, 1983; Holt et al., 1985; Saucier et al., 1992; Saucier and Baltz, 1993), we assumed that spawning began at 1800 h and stopped at 2200 h. Thus we were able to target spotted seatrout in the mid- to late afternoon specifically to capture females with oocytes in the late stages of FOM for batch fecundity (BF) estimation. Because we have consistently identified recently spawned females in shallow areas, they apparently return to the marsh edge where they once again become available for capture with our sampling gear. Our stratified random sampling of estuarine areas along the coast (described previously) was designed to representatively sample these recently spawned females for SF estimation.

We conducted BF sampling during two consecutive afternoons fortnightly from the middle of April through the first week of September 1998, 1999, and 2000. We deployed a trammel net from a shallow water boat as described above at preselected sites in Charleston Harbor in depths ranging from 1.0 to 1.5 meters during the afternoon (1400–1800 h EDT) high tide.

Restricting our sampling to the hours immediately preceding the evening spawning event ensured that those females preparing to spawn were available for capture. Male spotted seatrout, identified by their drumming sounds, caught during this targeted effort were measured and released at the site of capture. We supplemented samples for BF estimation with specimens from local sportfishing tournaments held during summer months in the Charleston Harbor area.

We processed samples in the laboratory as previously described. If ovaries appeared by macroscopic examination to contain hydrated oocytes, they were fixed in 10% buffered seawater formalin for potential counts (Hunter et al., 1985). The appropriateness of these ovaries for BF counts was subsequently determined by examining the corresponding histological preparation.

To ensure that only those oocytes destined to be ovulated during the upcoming spawning event were counted, we chose to use only those oocytes undergoing FOM that could be easily separated by size from late vitellogenic oocytes (Nieland et al., 2002; Lowerre-Barbieri et al.[8]). If we observed numerous recent POFs in the histological sample, the corresponding whole ovary was not used for oocyte counts (because their presence indicated that ovulation had occurred). We reweighed ovaries (approximately 2 weeks after fixation) to the nearest 0.01 g and randomly extracted three 130–150 mg aliquots from eight potential locations in the ovary (each lobe was partitioned into quarters lengthwise). We stored subsamples in 50% isopropyl and counted oocytes under a Nikon SMZ-U dissecting microscope at 12× magnification. We counted each subsample twice by using a Bogorov tray and a hand-held counter and conducted a third count if the two initial counts were dissimilar by more than 10%. We used the mean number of oocytes in each subsample to calculate mean oocyte density (number of oocytes per gram preserved ovary weight) and total numbers of oocytes in the ovary. We compared mean oocyte densities among the four regions of each ovarian lobe and between the two lobes by using a two-way analysis of variance (ANOVA). Because our variances were heteroscedastic, we used nonparametric ANOVA (Kruskal-Wallis or ANOVA on ranks) for comparisons of mean BF among ages, months, and years. To investigate the relationships between BF and length, somatic weight (ovary-free body weight), and age, we used linear regression.

Relative fecundity (RF) was calculated as the number of oocytes per gram somatic weight (ovary-free). To select samples for inclusion in RF calculations, we looked for the presence of nuclear migration in histological preparations. We used this criterion to ensure that oocytes of similar morphological dynamics would be used, minimizing the potential for error. We used the Kruskal-Wallis test to investigate the effect of age on RF. Because sample sizes were quite uneven among months, we chose to compare RF between the beginning and end of the spawning season (May and August). This comparison was done by using a Mann-Whitney test. To corroborate any trends in RF, we also conducted diameter measurements on the preserved (10% buffered

[7] Crabtree R. E., and D. H. Adams. 1998. Spawning and fecundity of spotted seatrout, *Cynoscion nebulosus*, in the Indian River Lagoon, Florida. *In* Investigations into nearshore and estuarine gamefish abundance, ecology, and life history in Florida, p. 526–566. Tech. Rep. for Fed. Aid in Sport Fish Rest. Act Project F-59. Florida Marine Research Institute, Department of Environmental Protection, 100 Eighth Ave. SE, St. Petersburg, FL 33701.

[8] Lowerre-Barbieri, S. K., L. R. Barbieri, and J. J. Albers. 1999. Reproductive parameters needed to evaluate recruitment overfishing of spotted seatrout in the southeastern U.S. Final report to the Saltonstall-Kennedy (S-K) Grant Program (grant no. NA77FD0074), 23 p.

seawater formalin) oocytes. We used a video camera mounted on a Nikon SMZ-U dissecting microscope and coupled to a PC equipped with a frame-grabber and with OPTIMAS® Image Analysis software (version 6, Media Cybernetics, Bothell, WA). Two readers independently measured the diameter of approximately 30 preserved oocytes in each of three subsamples from 27 ovaries. To test for uniformity of size throughout the ovary, mean oocyte diameters were compared between ovarian lobes and among subsample locations within each lobe by using two-way ANOVA. We also compared mean oocyte diameters among months and ages by using two-way ANOVA.

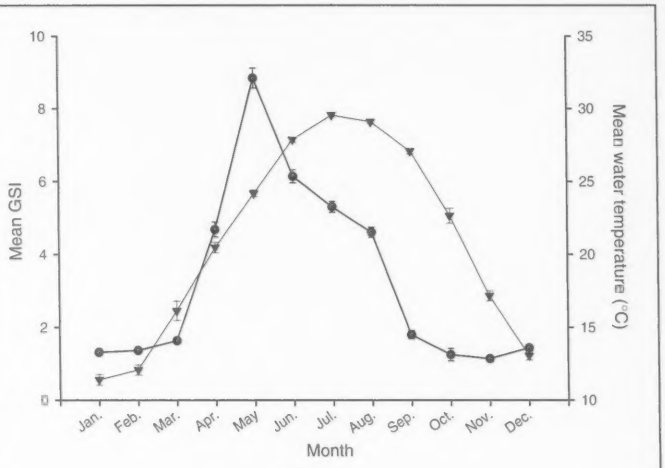

Figure 2

Mean monthly gonadosomatic index (GSI) for spotted seatrout in South Carolina for years 1991–2000 (circles). Mean water temperature for 1991–2000 (triangles). Error bars are standard errors. n = 1185.

Annual fecundity

Wiley (1996) demonstrated that spotted seatrout in South Carolina estuaries constitute a single population. Therefore, we felt justified in calculating monthly egg production (MEP) by multiplying the monthly SF (of specimens taken along the entire coast) by the mean monthly BF (of specimens from Charleston Harbor). Because not all age-1 female trout were mature at the beginning of the spawning season, the fraction of mature age-1 females obtained from previous work in South Carolina (Wenner[6]) was used to refine the MEP estimate. Because the latter was calculated by using SF obtained from data pooled across years, any comparison of MEP among years was deemed invalid. Kruskal-Wallis tests were used to determine whether MEP varied among months for each age class.

Monthly MEP estimates were summed to arrive at an annual fecundity (AF) estimate for each age class. Because the majority of individuals used in this study were aged 1–3, AF was estimated only for these age classes. We used linear regression to investigate the relationship between AF and age and thus predict AF for spotted seatrout aged 4 and 5. Using these predictions and the relative abundance of each age class in our samples, we estimated the contribution of each age class to the annual egg production.

All statistical analyses were conducted with the Statistical Package for the Social Sciences (version 9.0, SPSS Inc., Chicago, IL). The level of significance for all tests was 0.05.

Results

A total of 1038 spotted seatrout ranging in age from 1 to 5 was collected for this study. Because 97% of these belonged to age classes 1–3 we report reproductive parameters only for these ages. We examined a total of 941 mature and developing females, ranging in length from 248 mm to 542 mm TL, to determine spawning frequency (569, 285, and 87 for ages 1–3, respectively). Of these, 135 specimens (12 from sportfishing tournaments) were used to conduct oocyte counts (62, 52, and 21 for ages 1–3, respectively). These fish ranged in length from 268 to 530 mm TL. Minimum size at first maturity, as indicated by the presence of cortical alveoli stage oocytes in histological sections, was 248 mm TL. Size at 50% maturity was 268 mm, whereas 100% maturity was reached at 301 mm TL. Condition of females, as indicated by Fulton's condition factor, diminished over the course of the season (P<0.01, r^2=0.24).

Seasonality

Spawning in the Charleston Harbor area during the study period began in mid to late April as indicated by the presence of oocytes in late FOM or POFs in histological samples. During the study period, mean water temperatures ranged from 16° to 34°C. Highest temperatures were recorded during July and August for all three years of the study. The lowest documented water temperature when spawning began was 20°C. Cessation of spawning occurred when water temperature was 28°C. Mean monthly GSI for spotted seatrout captured along the South Carolina coast since 1991 (Fig. 2) showed a marked increase from 4.6 in April to 9.4 in May. Mean GSI in June declined to 6.3 and remained around 5.0 in July and August. A sharp decline was noted in September to 2.7, the lowest level for the season. Overall, mean gonadosomatic index (GSI) values followed the seasonal trend in water temperature (Fig. 2). Percent occurrence of females in spawning condition as evidenced from histological examination declined from approximately 87% in August to 12% in September. The percentage of

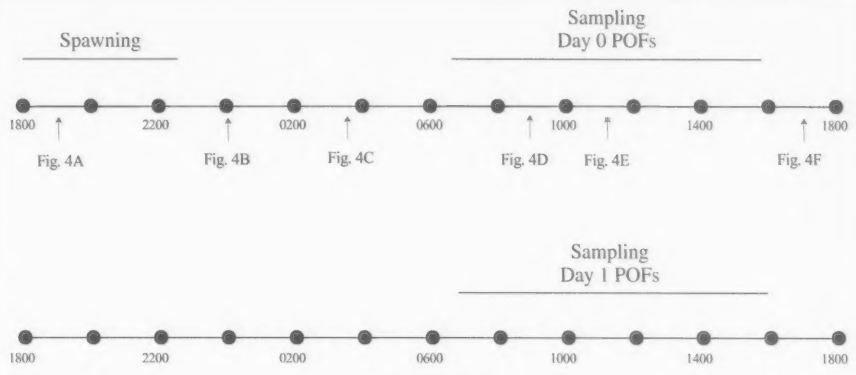

Figure 3

Forty-eight-hour chronological time line indicating *C. nebulosus* spawning time in Charleston Harbor, SC. Postovulatory follicles (POFs) observed at water temperatures >25°C were termed day-0 (<24 hours). Day-1 POFs (>24 hours) were observed only at water temperatures <25°C. Time of capture for specimens in Figure 4 are indicated.

post-spawning females increased from 9% in August to 91% in September. Thus, the spawning season for spotted seatrout in South Carolina extends from late April through early September.

Spawning frequency

Day-0 POFs were found through 1800 h of the day following a spawning event. Day-1 POFs were first observed in our routine samples when they were 36–37 hours old (the second day following a spawning event) only when water temperatures were below 25°C. Day-1 POFs were excluded from our analysis of SF because they did not provide evidence of a previous night's spawning event.

Figure 3 illustrates the time line for POF atrophy in spotted seatrout from 1–42 h after the onset of spawning at 1800 h. Because evidence of spawning for the first 12 h was documented only during a period when water temperatures were greater than 25°C, all of the examples shown are indicative of atrophy in warmer temperatures (Fig. 4). As indicated in Table 2, there was a time-dependent deterioration of POFs such that only those <24 h were detectable at water temperatures >25°C.

Small sample sizes prevented calculation of monthly SF for each age class by year. Therefore, we pooled data for all three years of this study to obtain a single monthly SF estimate by age class (Tables 3 and 4). The interaction between month and age on SF could not be statistically tested; however, age-3 fish spawned more frequently than younger fish (Kruskal-Wallis, $P<0.05$) and all seatrout spawned more frequently in June (Kruskal-Wallis, $P<0.05$). Peaks in SF observed for fish ages 2 and 3 in July and August, respectively (Tables 3 and 4), were not statistically significant.

Monthly SF values based on the occurrence of ovaries containing oocytes in FOM are also presented in Table 3. However, statistical comparisons were not feasible because of the nonrandom collection of specimens. Overall SF was estimated to be once every 4.4 days and once every 5.1 days with the POF and FOM methods, respectively.

Table 3

Spawning frequency (SF) expressed as the number of spawnings per month for *C. nebulosus* ages 1–3 for the spawning seasons of 1998–2000. Numbers in parentheses represent days between spawnings. *n* = number of fish in a sample. FOM = final oocyte maturation; POF = postovulatory follicle.

Age (yr)	Month	*n*	SF FOM method	SF POF method
1	May	89	4.53 (6.85)	4.18 (7.42)
	June	166	4.53 (6.62)	9.40 (3.13)
	July	185	4.68 (6.62)	6.54 (4.74)
	August	129	6.26 (4.95)	4.57 (6.79)
	Total	569	19.9 (6.18)	26.34 (4.67)
2	May	114	11.5 (2.78)	6.80 (4.56)
	June	79	5.70 (5.26)	7.60 (3.95)
	July	48	0.65 (47.62)	9.04 (3.43)
	August	44	9.87 (3.14)	6.34 (4.89)
	Total	285	30.67 (4.01)	29.36 (4.19)
3	May	46	10.10 (3.07)	7.42 (4.18)
	June	23	5.22 (5.75)	9.12 (3.29)
	July	10	3.10 (10.00)	3.10 (10.00)
	August	8	11.61 (2.67)	11.61 (2.67)
	Total	87	32.54 (2.67)	31.14 (3.95)
Overall		941	24.3 (5.06)	27.7 (4.44)

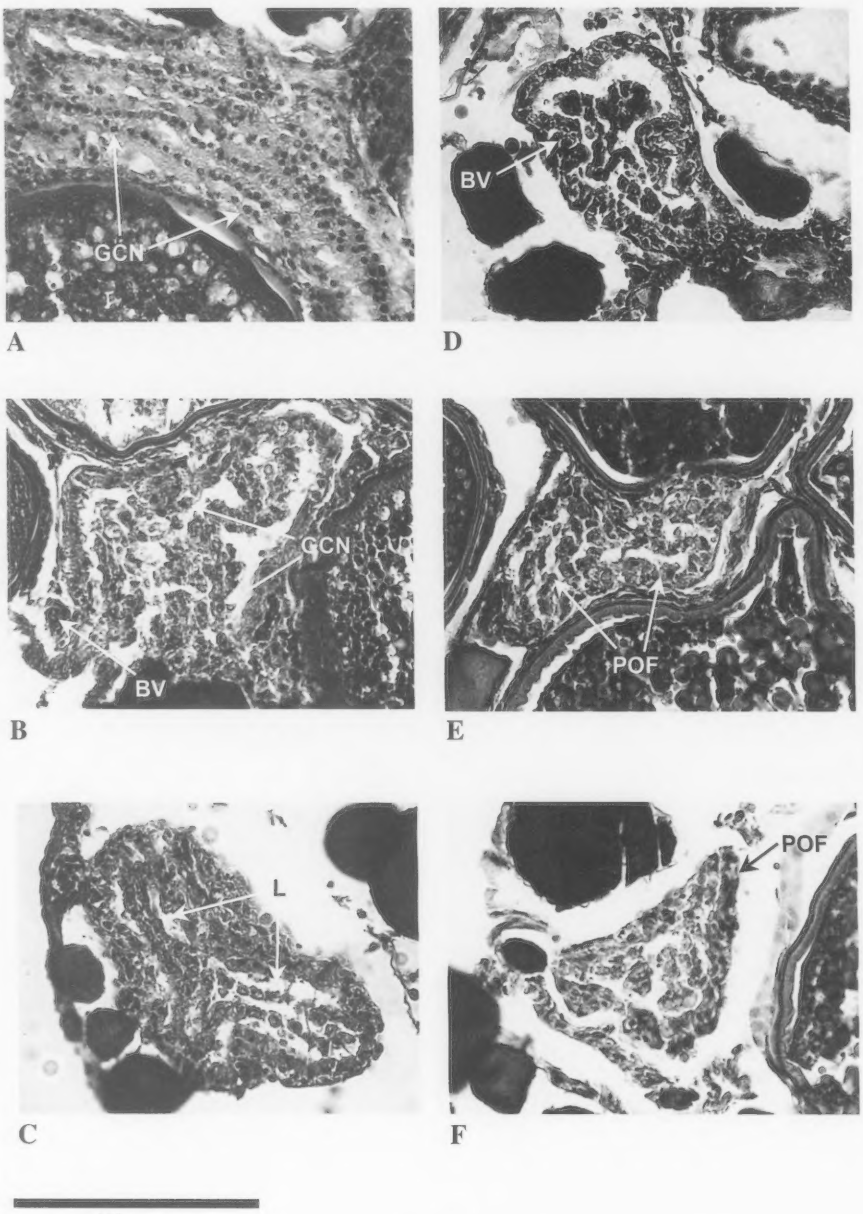

Figure 4

Photomicrographs of *C. nebulosus* postovulatory follicles (POFs) showing chronology of atresia at water temperatures >25°C. (**A**) 0–4 hours after spawning, (**B**) 5–8 hours after spawning, (**C**) 9–12 hours after spawning, (**D, E, F**) 13–24 hours after spawning.

Batch fecundity

As expected, we found a significant difference in mean BF among age classes (ANOVA on ranks, $P<0.05$). Age-1 spotted seatrout produced an average of 145,452 oocytes per batch spawned. Fish aged 2 and 3 spawned an average of 291,123 and 529,976 oocytes per batch, respectively. Therefore, mean BF was compared among months

Table 4

Fecundity parameters for *C. nebulosus* ages 1–3 from South Carolina estuaries. BF = batch fecundity in numbers of oocytes; SF = spawning frequency based on the postovulatory follicle (POF) method and expressed as the number of spawnings per month; MEP = monthly egg production = $(BF \times SF)\%mature$. Annual fecundity is the sum of mean monthly MEP values for each year class and represents the total number of oocytes produced by any given female from 1 May to 31 August. Numbers in parentheses indicate sample sizes.

Age (yr)	Month	Mean BF	SF	% mature	Mean MEP
1	May	117,760 (12)	4.18 (89)	78.6	386,897
	June	135,403 (16)	9.40 (166)	94.0	1,196,418
	July	141,237 (16)	6.54 (185)	97.0	895,978
	August	176,594 (18)	4.57 (129)	100	807,035
	Annual fecundity=3,286,328 oocytes				
2	May	280,724 (34)	6.80 (114)	100	1,908,926
	June	307,322 (10)	7.60 (79)	100	2,335,650
	July	370,170 (1)	9.04 (48)	100	3,346,337
	August	307,195 (7)	6.34 (44)	100	1,947,620
	Annual fecundity=9,538,533 oocytes				
3	May	487,475 (13)	7.42 (46)	100	3,617,061
	June	519,630 (4)	9.12 (23)	100	4,739,027
	July	765,911 (2)	3.1 (10)	100	2,374,325
	August	590,994 (2)	11.61 (8)	100	6,861,439
	Annual fecundity=17,591,852 oocytes				

Table 5

Monthly relative fecundity (number of oocytes /grams ovary-free weight) for *C. nebulosus* ages 1–3 for the spawning seasons 1998–2000. SD=standard deviation.

Month	Mean	Minimum	Maximum	SD	*n*
May	518.6	223.9	976.1	146.2	46
June	603.2	205.7	1306.1	241.8	20
July	820.9	662.2	1314.4	279.0	5
August	693.6	397.3	1021.8	207.9	12

and years for each age class separately (Table 4). There were no significant interannual or monthly variations in mean BF for any of the age classes (age-1: $P=0.59$, $n=62$; age-2: $P=0.17$, $n=52$; age-3: $P=0.07$, $n=21$). However, BF analysis for age-2 fish excluded the month of July because only one two-year-old specimen was captured that month during the study period. We investigated the relationship between BF and total length by using linear regression analysis. After pooling data across years, we found that total length explained 67% of the variability in spotted seatrout BF (Fig. 5A). Batch fecundity showed a similarly strong relationship to female somatic (ovary-free) weight (Fig. 5B) but did not relate to age as strongly (Fig. 5C). The equations below describe these relationships:

$BF = 2179.65(TL) - 520597$ $(r^2=0.67)$ $P<0.001$
$BF = 530.60(OFWT) + 18537.77$ $(r^2=0.65)$ $P<0.001$
$BF = 169398.21(Age) - 30956.33$ $(r^2=0.58)$ $P<0.001$.

Mean MEP was significantly different among months for age-1 spotted seatrout (Kruskal-Wallis, $P<0.05$). Age-1 fish spawned the least number of oocytes in May and most in June (Table 4). Statistical comparisons among months for ages 2 and 3 were inconclusive.

Relative fecundity

Relative fecundity among 83 spotted seatrout ages 1–3 ranged from 224 oocytes to 1314 oocytes/g OFWT (Table 5). Age did not have an effect on relative fecundity (Kruskal-Wallis, $P=0.75$). We found that spotted seatrout in South Carolina produced significantly more oocytes per gram ovary-free weight at the end than at the beginning of the spawning season (Mann Whitney, $P<0.05$). Mean oocyte diameters did not vary significantly between ovarian lobes or among locations within each lobe (ANOVA, $P=0.28$). A comparison among

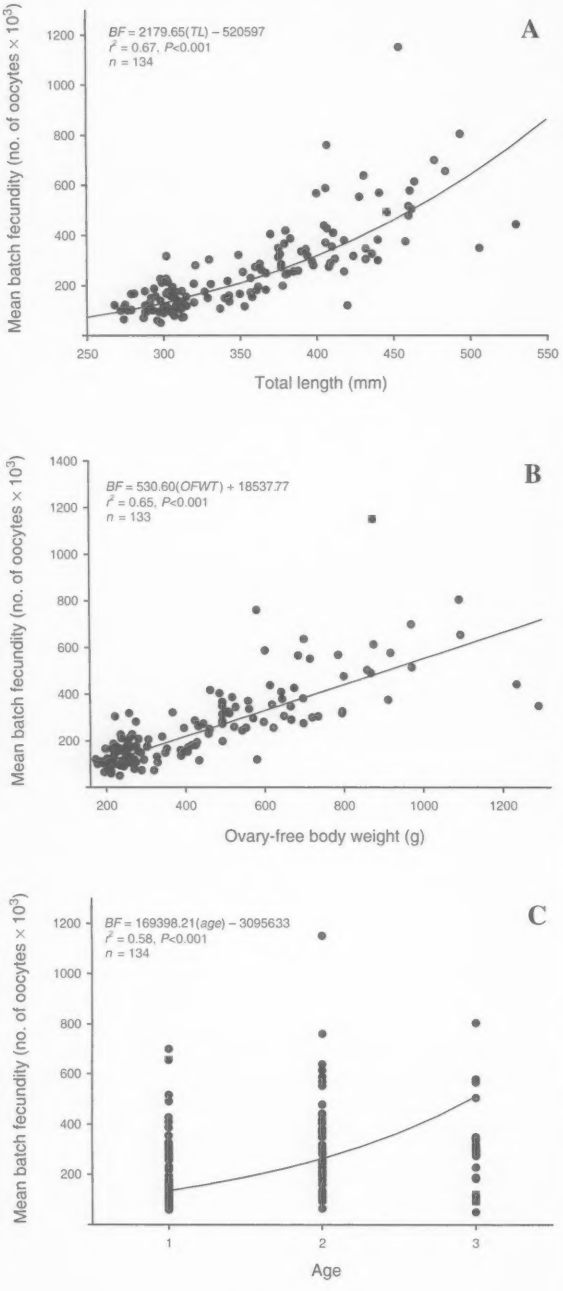

Figure 5

Relationship between batch fecundity (BF) and total length (TL) (**A**), between BF and ovary-free weight (OFWT) (**B**), and between BF and age (**C**) for *C. nebulosus* ages 1–3. Linear regression on data pooled for spawning seasons 1998–2000.

months and ages revealed that age had no effect on oocyte diameter (ANOVA, P=0.82). However, the effect of month corroborated the pattern of increasing RF as the spawning season progressed: oocytes were significantly smaller at the end of the season (ANOVA, P<0.05).

Annual fecundity

Annual fecundity estimates (summation of MEP) were approximately 3.2 million, 9.5 million, and 17.6 million oocytes for each age class, respectively (Table 4). The equation below describes the relationship between AF and age:

$$AF = 7152762(Age) - 4166620 \quad (r^2=0.99) \quad P<0.05.$$

From this relationship, the predicted AF for ages 4 and 5 were 24,444,430 and 31,597,190 oocytes, respectively. We expanded AF in relation to the abundance of each age class in our standard random samples for the three years of the study. We estimated that the overall average contribution from age-1 fish to the reproductive output for the season was approximately 29%, whereas fish aged 2 and 3 contributed 39% and 21% of oocytes, respectively. Ages 4–5 comprised less than 3% of specimens sampled and contributed 7% and 4% based on predicted AF values.

Discussion

Studies on the reproductive biology of *Cynoscion nebulosus* have established group-synchrony and indeterminate fecundity for this species throughout its range (i.e. Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001; Nieland et al., 2002; Mercer[1] and references therein). Fish with these features release gametes in several batches over a protracted spawning season and annual fecundity is not fixed prior to the onset of spawning (Wallace and Selman, 1981).

Based on mtDNA variation among spotted seatrout, the existence of two populations, one in the Gulf of Mexico and one in the South Atlantic, was established by Gold et al. (1999). However, variations in reproductive parameters have been suggested among geographic locations within the Gulf of Mexico (Brown-Peterson et al., 2002). Wiley (1996) suggested that spotted seatrout comprise a single stock in South Carolina; therefore reproductive parameters presented in the present study should be applicable only to the spotted seatrout population inhabiting coastal waters of this state. Further studies should be conducted to evaluate the applicability of these parameters to the entire southeast coast.

Other investigators (Brown-Peterson et al., 1988; Wieting, 1989; Brown-Peterson and War-

ren, 2001; Nieland et al., 2002; Lowerre-Barbieri et al.[8]) have used the gonadosomatic index (GSI) to delineate the spawning season in spotted seatrout. Even though the GSI provided a good approximation of the spawning season, histological data alone provided more precise evidence. Spotted seatrout in South Carolina began spawning near the end of April of each year and ceased by early September. Similarly, Lowerre-Barbieri et al.[8] reported that the spawning season for spotted seatrout in Georgia extended from late April to mid-September. We found histological evidence of initial spawning in specimens captured in 20°C water, although approximately 75% of spawning occurred when ambient water temperatures were greater than 25°C. In laboratory experiments, Brown-Peterson et al. (1988) found no successful spawning in water below 23°C but pointed out that others (McMichael and Peters, 1989) found eggs and larvae in 20.4°C water.

We found that females became mature approximately one full year after their birth. A female born in May of one year would be reproductively active in May of the following year. Females born later in the season would not be mature as the same successive season began; therefore, not all one-year-old females were mature when the spawning season began in May, but became mature before that season ended. This maturity schedule has also been reported for spotted seatrout in Louisiana (Nieland et al., 2002). However, Lowerre-Barbieri et al.[8] found that all one-year-old females were mature in coastal Georgia. A limited sample size or habitat segregation of mature and immature trout (Lowerre-Barbieri et al.[8]) may have contributed to their result.

The size at first maturity for spotted seatrout in this study was 248 mm TL. This size is comparable to what others have reported in other areas of the species' range (Brown-Peterson et al., 1988; Brown Peterson and Warren, 2001; Nieland et al., 2002; Mercer[1] and references therein; Lowerre-Barbieri et al.[8]). Our estimate of size at 50% maturity (268 mm TL) was larger than what Nieland et al. (2002) reported for 100% mature trout in Louisiana (250 mm TL). However, Nieland et al.'s (2002) statement that animals are 100% mature at 250 mm TL, does not agree with the growth equation they report for female trout when age = 1. Because we found size at 100% maturity among female spotted seatrout in South Carolina to be about 300 mm TL, we wonder whether Nieland et al.'s (2002) growth equation for female TL was meant to represent SL. Were this the case, they might have offered a different rationale for size at maturity among trout in Louisiana.

Brown-Peterson et al. (1988) and Brown-Peterson and Warren (2001) reported size at 100% maturity of 356 mm and 309 mm TL (using the SL-TL conversion found in our "Methods: section) for spotted seatrout in Texas and Mississippi, respectively. Brown-Peterson et al. (1988), however, chose a combination of gears that may not have sampled the trout population in Texas representatively for size-at-maturity estimation. In Mississippi, Brown-Peterson and Warren (2001) used a more appropriate gear for capture of late juvenile and early adult fish. Our estimate of size at 100% maturity was quite similar to theirs.

Spawning frequency

Determining the number of multiple spawning events during a single season for individual fish has been problematic. Initially, there was little understanding of the reproductive dynamics of spotted seatrout, and BF estimates were reported to represent the output for a whole season (Pearson, 1929; Sundararaj and Suttkus, 1962; Overstreet, 1983). Hunter et al. (1985) and Hunter and Macewicz (1985) developed techniques to overcome these limitations by providing protocols for the use of hydrated oocytes in determining BF and SF among group-synchronous species.

To use the techniques of Hunter (1985) and Hunter and Macewicz (1985) appropriately, it is critical to obtain a representative sample of the spawning population. DeMartini and Fountain (1981) and Lisovenko and Adrianov (1991) maintained that the relative occurrence of hydrated oocytes (as determined macroscopically) was an effective measurement of SF when the spawning population was sampled representatively. However, when sampling a species that spawns in aggregations at specific geographic locations, as do many of the sciaenids, it is inherently impossible to obtain a statistically representative sample of the spawning population for SF estimation based on FOM. Because the window of opportunity is temporally and spatially constrained, obtaining a sample that includes all sizes and ages involved is not feasible; the only choice in this situation is to sample in a directed fashion. This was the sampling strategy used to target females for BF counts; the majority of the animals captured whose oocytes evidenced FOM were obtained in a nonrandom fashion. Additionally, we assumed that fishes demonstrating FOM were moving toward deeper water spawning aggregations and away from our capture gear. For these reasons, we felt that our SF estimates based on the proportion of females with oocytes in FOM were biased and we excluded them from AF estimation. This is an important matter to keep in mind when comparing frequencies of spawning based on different methods.

Because obtaining representative numbers of animals with late-maturing oocytes is not often feasible, researchers have relied on the relative abundance of postovulatory follicles (POFs) to calculate SF (Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001; Nieland et al., 2002; Lowerre-Barbieri et al.[8]). The POF method lacks the limitations (described above) of the FOM method. Because the method we chose allowed us to sample all sizes and ages of fish in the estuary, obtaining representative numbers of animals with POFs was accomplished effectively. Therefore, we felt that our estimates of SF based on the POF method were more precise and we chose to use them in deriving AF.

The POF method depends on the ability to assess the disappearance of these structures. Hunter and Macewicz (1985) systematically sampled captive spawning anchovies to develop histological criteria for POF atrophy in

19°C water. Their criteria have been used by others to estimate rates of POF atrophy in other species and thereby determine the percentage of a population undergoing spawning over a discrete time period (Brown-Peterson et al., 1988; Fitzhugh et al., 1993; Taylor et al., 1998; Macchi and Acha, 2000; Brown-Peterson and Warren, 2001; Nieland et al., 2002). However, even though it has been demonstrated that the rate of POF atresia depends largely on ambient water temperature (Fitzhugh and Hettler, 1995), few (Brown-Peterson et al., 1988; Macchi and Acha, 2000; Nieland et al., 2002) have taken this into account when establishing the age of POFs for SF estimations. Our diurnal sampling of reproductively active spotted seatrout during warm water conditions enabled us to establish criteria to accurately estimate the age of POFs throughout the spawning season. Furthermore, we verified our assessments by sampling around the clock on two occasions to collect fish over the time period immediately following a spawning event.

Spotted seatrout ages 1–3 in SC spawned less frequently than those from the Indian River Lagoon, Florida (Crabtree and Adams[7]) but both studies showed that older fish spawned more frequently than younger animals. Our estimates for spotted seatrout aged 1–3 were 4.7, 4.2, and 4 days, respectively. Trout in these age classes in Florida were reported to spawn once every 4, 2.8, and 2.5 days, respectively. These differences probably not only reflect the distinct biological environments of each region but also indicate potential discrepancies in aging methods. No age-specific estimates of SF are available for other areas in the species' range. Brown-Peterson and Warren (2001) found SF among spotted seatrout in Biloxi Bay, MS, to be significantly lower than that of fish inhabiting the other two areas included in their study. They suggested that Biloxi Bay was a less conducive spawning habitat because of several factors, including shoreline development and a reduced amount of aquatic vegetation. However, because we found that SF varied significantly among age classes (age-3 fish spawned more frequently), the relative age composition of fish sampled by Brown-Peterson and Warren (2001) in the three estuaries might also have played a critical role in the determination of SF.

Batch fecundity

The best approach for estimating BF is to use only oocytes in FOM (Hunter et al., 1985; Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001; Brown-Peterson et al., 2002; Nieland et al., 2002; Lowerre-Barbieri et al.[8]). When it is not possible to obtain these, BF estimations can and have been carried out in some species by using the largest vitellogenic oocytes (Overstreet, 1983; Hunter et al., 1985; Wieting 1989). These efforts have the potential of being less accurate because isolating those oocytes destined to be spawned is difficult if the latter have not yet reached final maturation (Nieland et al., 2002). Inevitably this scenario would result in a nonmeasurable overestimation of female reproductive output. Brown-Peterson et al. (1988) and Brown-Peterson and Warren (2001) used a modification of this approach to estimate BF of spotted seatrout in Texas and Mississippi, respectively. However, even though the potential existed for overestimating BF, their estimates fell well below those presented in the present study, as did those presented by Nieland et al. (2002) for spotted seatrout ages 2–4 in Barataria Bay, Louisiana. Mean BF for ages 1–3 (170 thousand, 226 thousand, and 274 thousand oocytes, respectively) spotted seatrout in Indian River Lagoon, Florida (Crabtree and Adams[7]), also differed from those reported here. Our estimate took into account that not all age-1 females were mature at the beginning of the season. Crabtree and Adams,[7] however, did not adjust their estimate to reflect this discrepancy. Moreover, due to differences in aging methods, their age-1 and 2 cohorts possibly included ages 2 and 3, respectively. In addition, in the Florida study as well as in ours, relatively few numbers of older specimens were examined.

The relationships between BF and length, weight, and age in the present study were significant and predictive. Of these, TL exhibited the most predictive relationship. This fact may explain why age-1 and age-2 spotted seatrout in Georgia had mean BFs considerably higher than ours (175 thousand and 407 thousand, respectively; Lowerre-Barbieri et al.[8]): the size ranges for age-1 and age-2 in the Georgia study were greater than ours. Total length seems to be the most reliable predictor of BF among spotted seatrout in Georgia and SC (Lowerre-Barbieri et al.,[8] this study) and in Louisiana (Nieland et al., 2002). However, Crabtree and Adams[7] found that BF related best to ovary-free weight among spotted seatrout in Florida. We found ovary-free weight to be the second best predictor of BF. Overall, it appeared that TL and ovary-free weight were better predictors of BF than age for this species (Brown-Peterson, 2003).

As with SF, monthly egg production (MEP) estimates for SC spotted seatrout varied throughout the season. Because BF was not significantly different among months for any of our age classes, the variation in MEP resulted directly from the frequency of spawning. Monthly egg production estimates for age-1 fish were lowest in May and highest in June because SF was lowest in May and highest in June. Spawning frequency is a critical reproductive parameter because it seems to dictate annual reproductive output (DeMartini and Fountain, 1981; Brown-Peterson and Warren, 2001; Crabtree and Adams[7]); therefore, SF should be carefully considered, particularly for managed species.

Relative fecundity

We found that relative fecundity (RF), the number of oocytes per gram of somatic weight, did not show a significant relationship with female size. This finding was expected because dividing fecundity by ovary-free weight standardizes the values independently of size. However, this finding was in contrast to that of Brown-Peterson and Warren (2001). They collected specimens during the morning only, whereas we sampled ours throughout the day. This procedure allowed us to examine ovaries

over the entire range of maturation and to select only those clearly showing nuclear migration (based on histological observations) ensuring that only oocytes in the same phase of FOM (Brown-Peterson et al., 1988) were included in RF calculations. If sampling is conducted during a time period that is not close to active spawning (i.e., when oocytes are in different phases of FOM), then the number of oocytes per gram may be miscalculated.

As with BF, our RF estimates were higher than those reported for seatrout in the Gulf of Mexico (Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001), although spotted seatrout reproductive parameters appeared to vary considerably even within the Gulf of Mexico (Brown-Peterson et al., 2002). This was attributed to differential environmental conditions or food availability (or to both) (Brown-Peterson and Warren, 2001; Brown-Peterson et al., 2002). The significant seasonal increase in RF that we observed for spotted seatrout in South Carolina, however, has not been reported elsewhere. Brown-Peterson et al. (1988) found no differences in mean monthly RF among spotted seatrout in Texas. Brown-Peterson and Warren (2001) found significantly higher RF values in June than in August. In both instances, however, a small sample size may have biased their results.

Comparisons of mean oocyte diameters among months related the increase in RF to a general decrease in oocyte size over the course of the season. This phenomenon is widespread among marine pelagic spawners, and scientists have put forth several explanations to account for it (see Chambers, 1997). Bagenal (1971) suggested that egg size decreased over the spawning season owing to concurrent increased food availability for larvae. Others have suggested an inverse relationship between temperature and egg size (Ware, 1975; Wootton, 1994; Miller et al., 1995) or a seasonal decrease in egg size that is correlated to the condition of spawning females (DeMartini and Fountain, 1981; Chambers and Waiwood, 1996). The latter seems to apply to spotted seatrout in this study because a diminishing trend through the spawning season was observed in the condition factor of females.

Annual fecundity

Brown-Peterson (2003) presented AF estimates for spotted seatrout throughout their range. Our estimates were substantially below those for spotted seatrout in Indian River Lagoon (Crabtree and Adams[7]) but approximated those of Lowerre-Barbieri et al.[8] for trout in Georgia. A possible reason for the higher values in Florida was the more protacted spawning season in that area (50 days longer). No comparisons of AF estimates presented in this study and those of spotted seatrout in the Gulf of Mexico (Brown-Peterson, 2003) were made because they were not specific to age classes.

The main impetus behind the present study was to establish annual fecundity (AF) estimates by age class. We found that age-1 through age-3 spotted seatrout occurred abundantly in SC estuaries and that each of these age cohorts showed unique fecundity dynamics. The AF for an average age-1 fish was one-third that of age-2 (~3.28 million vs. 9.5 million). One year-old fish, however, constituted the majority of fish in our samples; their abundance was twice that of 2-year-olds and seven times that of 3-year-old fish. Even though the average age-3 trout produced almost twice as many oocytes during the season (17.5 million) as the average age-2 fish, their reduced abundance in our estuaries made their overall contribution only half that of 2 year-olds. Ages 4 and 5 were estimated to produce approximately 24.4 million and 31.6 million oocytes per female, respectively; however, the oocyte production by the predominant age groups overshadowed theirs. When analyzed in relation to the occurrence of the other age classes in our estuaries, age-2 fish contributed the greatest number of fertilizable oocytes to the environment (39%).

Reliable fecundities based on age and on length are optimal for stock assessment models (Williams[9]). This study provided AF estimates for three age classes that can be used in age-based models for the spotted seatrout population in South Carolina. Annual fecundity estimates based on length, however, have not been attempted even though length appears to be the best predictor of fecundity in spotted seatrout (see references in Brown-Peterson, 2003). Further analyses to investigate the relationship between egg production and fish length for each month of the spawning season would allow for more precise management efforts based on individual length-based estimates of AF.

Acknowledgments

We thank members of the Inshore Fisheries Section of the South Carolina Department of Natural Resources for assisting in field data collection throughout this study (C. Wenner, J. Archambault, H. von Kolnitz, W. Hegler, E. Levesque, L. Goss, C. McDonough, C. Johnson, A. Palmer). C. Wenner, H. von Kolnitz, and E. Levesque conducted age assessments. Histological processing was provided by C. McDonough, R. Evitt, A. Palmer, and W. Hegler. Assistance with oocyte counts was provided by C. McDonough, T. Piper, K. Maynard, and R. Evitt. Data management was coordinated by J. Archambault, C. Wenner, E. Levesque, and three anonymous reviewers provided helpful suggestions on the manuscript. Funding for this study was provided by the National Marine Fisheries Service under MARFIN grant no. NA77FF0550.

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Abstract—Dungeness crabs (*Cancer magister*) were sampled with commercial pots and counted by scuba divers on benthic transects at eight sites near Glacier Bay, Alaska. Catch per unit of effort (CPUE) from pots was compared to the density estimates from dives to evaluate the bias and power of the two techniques. Yearly sampling was conducted in two seasons: April and September, from 1992 to 2000. Male CPUE estimates from pots were significantly lower in April than in the following September; a step-wise regression demonstrated that season accounted for more of the variation in male CPUE than did temperature. In both April and September, pot sampling was significantly biased against females. When females were categorized as ovigerous and nonovigerous, it was clear that ovigerous females accounted for the majority of the bias because pots were not biased against nonovigerous females. We compared the power of pots and dive transects in detecting trends in populations and found that pots had much higher power than dive transects. Despite their low power, the dive transects were very useful for detecting bias in our pot sampling and in identifying the optimal times of year to sample so that pot bias could be avoided.

Estimating Dungeness crab (*Cancer magister*) abundance: crab pots and dive transects compared

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Manuscript submitted 13 March 2000 to Scientific Editor's Office.
Manuscript approved for publication 25 March 2004 by the Scientific Editor.
Fish. Bull. 102:488–497 (2004).

Reliable population assessments are fundamental to the management and conservation of commercially harvested crabs. Many crab populations are sampled with commercial crab pots to estimate population trends, to set harvest quotas, or to differentiate natural population fluctuations caused by anthropogenic changes to the ecosystem. Pots are used, for example, to assess the population status of blue crabs, *Callinectes sapidus* (Abbe and Stagg, 1996), red king crabs, *Paralithodes camtschaticus* (Zheng et al., 1993), snow crabs, *Chionoecetes opilio* (Dawe et al., 1996), and southern king crabs, *Lithodes santolla* (Wyngaard and Iorio, 1996).

The Dungeness crab (*Cancer magister*) fishery began in southeastern Alaska in 1916 and has been characterized by large fluctuations on annual and decadal scales (Orensanz et al., 1998). Large variation in the Dungeness crab harvest is not unique to Alaska; similar fluctuations have been documented in California and their causes are the subject of an ongoing debate (Higgins et al., 1997a, 1997b). It is not clear whether the processes that cause fluctuations in California are the same as those responsible for oscillations in Dungeness crab abundance in Alaska.

Most of the Dungeness crab fisheries in Alaska are managed by regulating the size and sex of the crabs caught, and, in some places, the season of the harvest. In southeastern Alaska, legal harvest is restricted to males with a carapace width greater than or equal to 165 mm (excluding the 10th anteriolateral spines) and the season is timed to avoid sensitive life

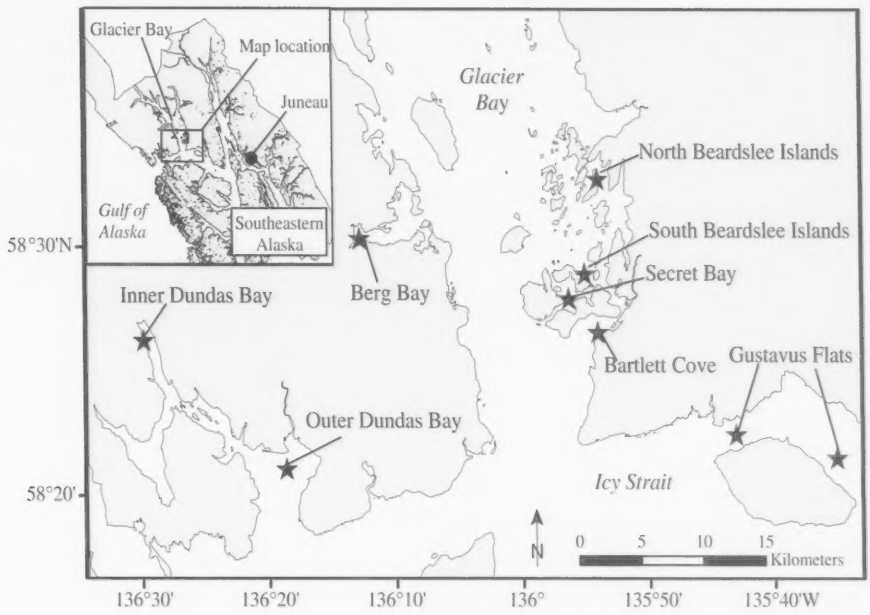

Figure 1

Map of study area showing eight study sites in or near Glacier Bay in southeastern Alaska.

history periods such as mating and molting (Kruse, 1993; Orensanz et al., 1998). Pre- and postseason stock assessment surveys using crab pots were initiated in southeastern Alaska in 2000 (Rumble and Bishop, 2002). The purpose of the latter management strategy is to assess the abundance of legal-size males before the fishing season, to estimate harvest rates, to define the timing of male and female mating and molting and to determine growth rate by tagging crabs.

The usefulness of surveys with pots for Dungeness crab population assessment, however, depends on the accuracy of these surveys in measuring population parameters. Factors that can bias catch per unit of effort (CPUE) and size-frequency estimates for Dungeness crabs are pot soak-time (Miller, 1974; High, 1976; Gotshall, 1978; Smith and Jamieson, 1989); freshness of bait (High, 1976; Smith and Jamieson, 1989); pot design (Miller, 1974; High, 1976; Smith and Jamieson, 1989); and agonistic interactions among conspecifics inside and at the entrance of pots (Caddy, 1979; Smith and Jamieson, 1989). Smith and Jamieson (1989) developed a standardized model to compensate for the effect of agonistic interactions, age of bait, and escapement. They also concluded that researchers could minimize these biases by measuring CPUE with standardized surveys with short soak times. These studies measured sampling bias with pots by comparing catch in pots among various experimental treatments. Opportunities for comparing surveys with pots to direct measures of abundance are rare. In our study, we compared the bias and power of CPUE estimates from surveys with pots to independent measures of abundance conducted by scuba divers on benthic dive transects.

Methods

Study area

The study area included eight sites in southeastern Alaska, near Glacier Bay: North Beardslee Islands (58°33′N 135°54′W), South Beardslee Islands (58°30′N 135°55′W), Berg Bay (58°31′N 136°13′W), Bartlett Cove (58°27′N 135°53′W), Gustavus Flats (58°23′N 135°43′W), Secret Bay (58°29′N 135°56′W), inner Dundas Bay (58°27′N 136°31′W), and outer Dundas Bay (58°21′N 136°18′W) (Fig. 1). All study sites were located within Glacier Bay National Park and Preserve, with the exception of Gustavus Flats, which was located adjacent to the Park boundary in Icy Strait.

Glacier Bay is a large (1312 km^2) glacial fjord system with high sedimentation rates of clay-silt particles from streams and tidewater glaciers (Cowan et al., 1988). The primarily unconsolidated rocky coastline is highly convoluted with numerous small bays. Dungeness crabs can be found throughout Glacier Bay; however the majority of the population are found in the lower 40 km of the estuary where our sites were located (Taggart et al., 2003). The shallow water in and around our study sites was primarily characterized by mud bottom, but sand, pebble, cobble, and shell substrates were also common (Scheding et al., 2001).

Table 1

Sampling dates for yearly spring and fall pot and dive surveys of Dungeness crabs (*Cancer magister*) in Glacier Bay, Alaska. Sample size (*n*) is listed for pots and dives for each sampling event.

	Spring sampling				Fall sampling			
Year	Pots	*n*	Dives	*n*	Pots	*n*	Dives	*n*
1992	7–12 April	248	7–12 April	69	17–22 Sept.	250	17–22 Sep.	75
1993	20–27 April	350	20–27 April	105	23–28 Sept.	250	23–28 Sep.	75
1994	20–27 April	350	23 April–1 May	105	13–18 Sept.	249	13–18 Sept.	75
1995	19–26 April	350	23 April–1 May	105	9–14 Sept.	236	15–19 Sept.	75
1996	15–21 April	350	22–28 April	105	13–18 Sept.	242	19–23 Sept.	73
1997	17–22 April	300	23–28 April	115	14–19 Sept.	298	20–25 Sept.	120
1998	—	—	—	—	9–14 Sept.	296	16–21 Sept.	91
1999	—	—	—	—	9–14 Sept.	299	17–22 Sept.	107
2000	—	—	—	—	9–14 Sept.	297	18–23 Sept.	60

Sampling dates

Sampling was conducted biannually, in April and September, from 1992 to 1997 and annually, in September, from 1998 to 2000 (Table 1). The spring and fall sampling periods were selected to coincide with crab life history events and to avoid sampling during commercial fishery operations. April sampling was scheduled to occur before larval hatching in May–June (Shirley et al., 1987) and before the summer commercial fishing season from 15 June to 15 August. September sampling began after the end of the fishing season (15 August) and ended before the beginning of the winter harvest (1 October to 30 November).

During 1992, the study sites were sampled with pots (referred to as "pot sampling") and by divers (referred to as "dive sampling") concurrently (Table 1). In 1993 and 1994, sampling was conducted on nearby study sites and the dive sampling usually one day ahead of the pot sampling. For logistical reasons, starting in 1995, we separated the pot sampling and the dive-transect sampling into two separate research cruises. The pot sampling was conducted on the first cruise and the dive sampling occurred on the second cruise; pot and dive sampling were separated at each location by 2 to 12 days.

Sampling with pots

Crabs were sampled with commercial crab pots (0.91 m in diameter, 0.36 m tall, with 5-cm wire mesh). Escape rings were sealed with webbing on each pot to retain smaller crabs. Pots were baited with hanging bait comprising salmon, cod, or halibut (depending on availability) and bait jars that were filled with chopped herring and squid. We found that cod was predictably available; therefore from 1996 on, we consistently used cod for hanging bait. Pots were soaked for 24 hours.

Within each study site, we set 25 pots in shallow water (0–9 m) and 25 pots in deep water (10–25 m). Each day we set 50 pots in one of the study sites and retrieved the 50 pots that had been set the previous day at one of the other study sites. The pots were set along strings parallel to shore at intervals of approximately 100 m. Within each study area, the strings of pots were located in prime Dungeness crab habitat determined by a local fisherman. We placed the pots at the same locations during subsequent sampling events by using a GPS (Rockwell PLGR+) with an accuracy of ±3 m. We estimate that the pots were set within 20 meters from the original waypoints. Water depth (standardized to mean lower low water), set and retrieval time, and GPS location were recorded for each pot. Water temperature and salinity profiles were measured at each study site during each sampling period with a SEABIRD SBE-19 Profiler.

As the pots were retrieved, we counted and identified all organisms. For all Dungeness crabs we recorded the sex, carapace width, shell condition, and damage to appendages. For female crabs we also recorded reproductive status. Carapace width was measured to the nearest millimeter immediately anterior to the 10th anterolateral spine with vernier calipers (Shirley and Shirley, 1988; Shirley et al., 1996). All organisms were returned to the water at the location where they were caught. A potential problem with returning the crabs to the water near the site of capture is the possibility that crabs could be resampled in subsequent pots, which would bias the catch per unit of effort. Beginning in April, 1995, all crabs collected in the South Beardslee Islands and Berg Bay were tagged with a sequentially numbered, double-T Floy tag (Floy Tag and Manufacturing Company, Seattle, WA) inserted along the posterolateral margin of the epimeral suture. Tags placed in this location are retained through ecdysis (Smith and Jamieson, 1989). Of the 5226 crabs tagged, only a single

crab was recovered during the same sampling event. Thus, the probability of resampling crabs by returning them to the water was very low.

Sampling by divers

Divers using scuba equipment censused crabs on 15 to 20, 2×100 m belt transects within each study site. Approximately one day of sampling was required at each study site. The dive transects were conducted perpendicular to the shoreline and they extended from the shallow subtidal (0 m, mean lower low water) to 18 m depth or to the end of the 100 m transect, whichever came first. Divers did not go below 18 m depth in an effort to reduce nitrogen accumulation in divers' blood and to reduce the surface intervals required between transects. From 1992 to 1997, transect locations were randomly selected in the same areas as the crab-pot sampling. The random locations selected in 1997 were resampled during the following years of the study.

Divers counted all Dungeness crabs located within 1 m of each side of the transect. An effort was made to locate buried crabs by swimming close to the bottom and looking for irregularities in the bottom or protruding crab eyestalks. Each crab was examined and the following were recorded: legal males ≥165 mm carapace width), sublegal males (<165 mm carapace width), ovigerous females, and nonovigerous females.

Data analysis

For each year, we calculated the average pot CPUE for each site by reproductive class (males, nonovigerous females, and ovigerous females). The number of pots sometimes deviated from 50 when a pot was lost or when the degradable cotton string securing the pot lid broke (range: 44–50 pots). The number of crabs counted on dive transects was averaged for each reproductive class by site for each year. All dive transects were conducted perpendicular to shore; thus the transects crossed the shallow habitat where the shallow string of pots was set and terminated at 18 m which was the center of the depth we targeted for the deep pot set. Because the deep pot set was at or slightly beyond the deep end of the transect, we may have sampled more crabs from deepwater habitats than from the shallower transects. However, we did not think this was a significant bias because we sampled crabs from a relatively large area. We, therefore, pooled the pots from both depth strata for analysis.

We tested for differences between April and September for the pot CPUE data and the dive density data with paired *t*-tests. CPUE and density data were not normally distributed; therefore we transformed the data with a square-root transformation $[Y=\sqrt{(Y+3/8)}]$ for statistical analyses (Zar, 1996). These analyses were conducted for males, nonovigerous females, and ovigerous females. Because seasonal increases in water temperature could drive differences in CPUE between April and September, we calculated mean water temperatures by averaging the water temperatures at the 5 m and 15 m depths at each site and year. This analysis was limited to years and sites where we collected samples in both April and September (1992–97, from five sites: North Beardslee Islands, South Beardslee Islands, Berg Bay, Bartlett Cove, and Gustavus Flats). We assessed how CPUE was influenced by two independent variables, water temperature and season, with stepwise regression. Because CPUE declined from 1992 to 1997 (Taggart et al., in press), we controlled for year so that it would not confound our analysis.

In order to assess sampling bias between pots and dive transects, the percentages of females (females/all crabs), nonovigerous females (nonovigerous females/all crabs), and ovigerous females (ovigerous females/all crabs) were calculated for each site and sampling time. We also compared the percentage of the male population that was legal size (legal-size male crabs/all male crabs) from the pots and from the dives. The percentage estimates from the pot data were compared to estimates from the dive transects with a paired sign test (Zar, 1996). If percentage estimates for pot data were unbiased when compared to estimates from dive data, the pot percentage estimates would have an equal chance of being higher or lower than the percentage estimates for the dive data. Because small sample sizes exaggerate percentage comparisons, we excluded samples where the total number of crabs collected was less than 25 crabs/site.

The power of pots and dive transects to detect trends in populations was compared with Monitor, a power analysis program (Gibbs and Melvin, 1997; Gibbs, 1998). For our analyses, we varied the number of transects and pots, compared males and nonovigerous females, and varied the duration of the study. For all analyses the following input parameters of the model were held constant: "survey occasions" = annual, "type" = linear, "significance level" = 0.05, "number of tails" = 2, "constant added" = 1, "trend variation" = 0, "rounding" = decimal, "trend coverage" = complete, and "replications" = 10,000.

To estimate power, the model requires "count" and "variance" for each plot across years for at least three years. Pot and transect data collected from 1992 to 1998 from five sites (North Beardslee Islands, South Beardslee Islands, Berg Bay, Bartlett Cove, and Gustavus Flats) were used for these analyses. The data were limited to September to avoid seasonal bias. The average across years was calculated for each transect and each pot. These averages were input into the model's variable called "plot count." For each pot and transect a linear regression was calculated among years (CPUE vs. year for pots; density vs. year for dive transects) and the residual mean square was the "plot variance" variable (Thomas and Krebs, 1997).

To estimate the effect of sample size on power we set the "number [surveys] conducted" to four and limited the analysis to males. We varied the number of "plots" (pots and transects). For pots, we randomly selected subsamples of the 250 pots and ran simulations from 25 pots to 250 pots in 25-pot increments. The number

of dive transects for which data were collected for multiple years was 75. For simulations with a sample size less than 75, we randomly subsampled the data in the same manner as we did with pots. For simulations with sample sizes greater than 75, we amplified the samples with simple bootstrapping to obtain samples from 100 to 250 transects in 25-transect increments (Wonnacott and Wonnacott, 1990). For each sample size, we modeled three annual rates of change (0.02, 0.03, and 0.05).

To evaluate how study duration affects power, we limited the analysis to males, varied study duration ("number [surveys] conducted") from two years to 12 years in two-year increments, and compared three annual rates of change (0.02, 0.03, and 0.05) for both pots and transects. To hold effort constant between the two sampling techniques, we set the pot and transect sample size to the number we could accomplish in a five-day research cruise (250 pots and 75 transects).

To explore the relationship between annual trend in population and power, we held effort constant (250 pots and 75 transects) and varied the annual trend (from −0.10 to +0.10 in 0.01 increments) for both males and nonovigerous females. It was not possible to conduct a power analysis for ovigerous females because a large proportion of the pots and transects had no ovigerous female crabs.

Results

The pot CPUE estimates for males, nonovigerous females, and ovigerous females was significantly different in April than in the following September (Fig. 2, A, C, and E). Male and nonovigerous female CPUE was higher in September (Fig. 2, A and C) and ovigerous female CPUE was lower in September (Fig. 2E). In contrast, April density estimates from dive transects were not significantly different from the following September density estimates for males (Fig. 2B). Dive density estimates for nonovigerous females were higher in September than in April (Fig. 2D); density estimates for ovigerous females were lower in September than in April (Fig. 2F).

When we tested the influence of temperature and season on male CPUE with stepwise regression, season was selected first; temperature was not selected because it did not have a significant additional effect (Table 2). Because no significant difference was found between the April and September density estimates from dive transects (Fig. 2B), we did not conduct a stepwise regression for the dive data.

Percentage estimates of females from sampling with pots were lower than percentage estimates from dive transects for a significant number of samples for both April and September (Fig. 3A); therefore pots were biased against sampling females. When females were split by reproductive status, no bias was detected for sampling nonovigerous females with pots (Fig. 3B). In contrast, the percentage estimates for ovigerous females remained biased and the magnitude of the bias increased (Fig. 3C). To test potential sampling bias related to crab size, we compared the proportion of the male population that was legal size sampled with pots and dives (Fig. 4). There was no significant bias when pots and transects were compared with a sign test (April, $P>0.999$; September, $P=0.06$).

CPUE estimates from pots had a higher power than density estimates from dive transects for the same sample size (Fig. 5). Because more time is required to conduct a dive transect than to set and pull a crab pot, the power of transects compared to pots was even lower when effort was incorporated into the analysis (Fig 6). The power can be increased for both pots and

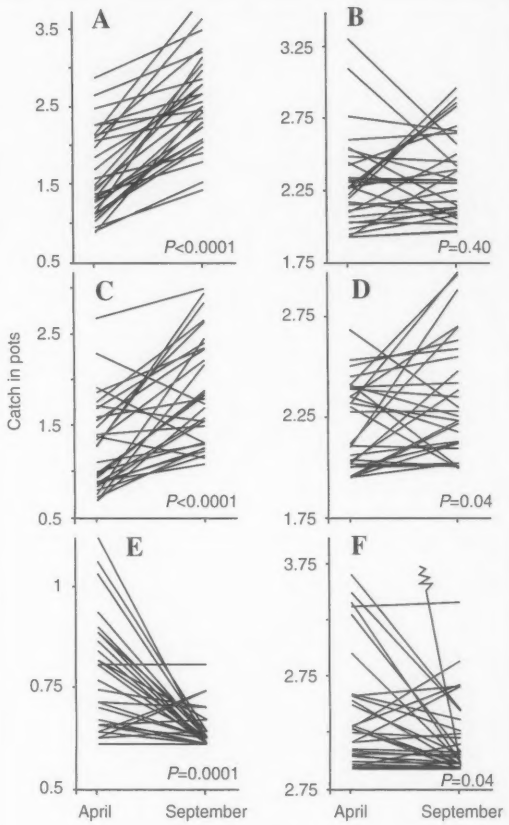

Figure 2

Within-year paired comparisons by site of catch in pots (left column) and density on dive transects (right column) for: (**A** and **B**) male Dungeness crabs (*Cancer magister*); (**C** and **D**) nonovigerous female crabs; and (**E** and **F**) ovigerous female crabs. Catch and density data were transformed with a square-root transformation. *P*-values indicate results from paired *t*-tests and significant results show differences between April and September. Lines on the graphs are parallel if measurements at sites were consistently higher or lower in April and September.

Table 2

Stepwise regression results of CPUE (male crabs/pot) versus three independent variables (year, season, and temperature).

Step	Model parameters	r^2	*P*-value (parameter)
1	*Year*	0.1493	0.001 (year)
2	*Year* and *month*	0.5589	0.04 (month)
3	*Year, month,* and *temperature*	0.5589	0.98 (temperature)

transects by increasing the study duration or increasing the amount of change in the population that the study is attempting to detect (Fig. 6). Although pots had more power than dive transects, there was only slightly more power to detect change in abundance of male crabs versus nonovigerous females (Fig. 7).

Discussion

For male Dungeness crabs, the density estimates from the dive transects showed no difference between April and September (Fig. 2B). The male CPUE estimates from pots, however, were consistently lower in April than in the following September (Fig. 2A). Because feeding rates of Dungeness crabs are correlated with temperature (Kondzela and Shirley, 1993), we thought that temperature was likely to explain the differences in CPUE between April and September. We found, however, that season had a larger effect than temperature (Table 2). This result suggests that seasonal factors other than temperature influence catchability. Stone and O'Clair (2001) followed the seasonal movements of Dungeness crabs in a glacial estuary in southeastern Alaska and reported that mean movement of male crabs was lower during the spring than in the late summer and fall. It is possible that our spring sampling schedule coincided with low male activity and male crabs were less likely to encounter a bait plume and be attracted to a pot. These results indicate that if pots are used for sampling, late summer and early fall is the time of year to conduct population assessment surveys of male crabs. Similar seasonal differences in CPUE have also been described for edible crabs (*Cancer pagurus*) and American lobsters (*Homarus americanus*) (Bennett, 1974). These data demonstrate the importance of controlling for season when comparing CPUE among years or sites.

The proportion of large crabs caught in pots increased with longer soak time for Dungeness crabs in British Columbia (Smith and Jamieson, 1989) and red king crabs in Britstol Bay, Alaska (Pengilly and Tracy, 1998). We found no bias when we measured the legal-size proportion of the male population caught in pots and compared it to the proportion sampled on dives (Fig. 4). We expect, however, that the bias observed in British Columbia and Bristol Bay would occur for our study sites if the soak time of pots were increased.

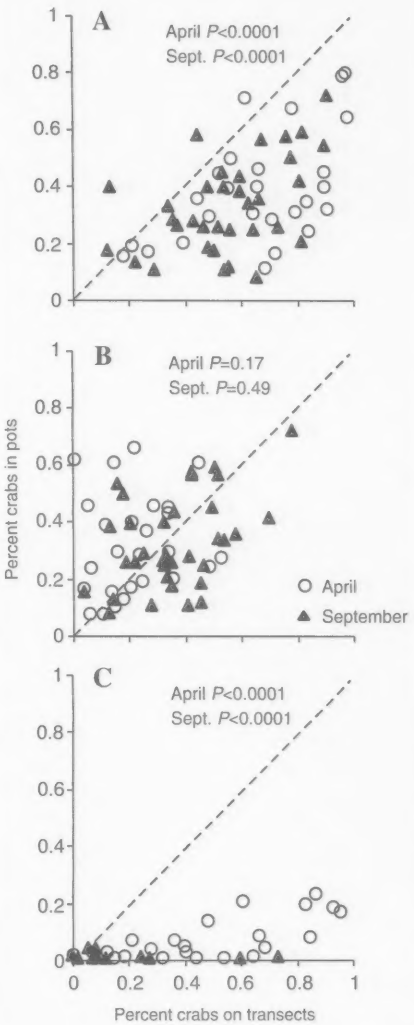

Figure 3

The percentage of (**A**) female Dungeness crabs (*C. magister*), (**B**) nonovigerous female crabs, and (**C**) ovigerous female crabs estimated from pots and from dive transects. The dashed line in each graph has a slope of 1; thus half of the data points should be above and half should be below the dashed line if percentage estimates for dives and pots are unbiased. Pot and dive transect data for each sex class and season were compared with a paired sign test and *P*-values are reported.

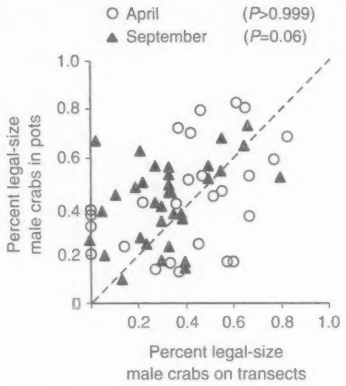

Figure 4

The percentage of male crabs of the Dungeness crab (*C. magister*) population that were legal size (≥165 mm) estimated from pots compared to the percentage of male crabs estimated from dive transects. Data from pots and dive transects were compared for each season with a paired sign test and *P*-values are reported.

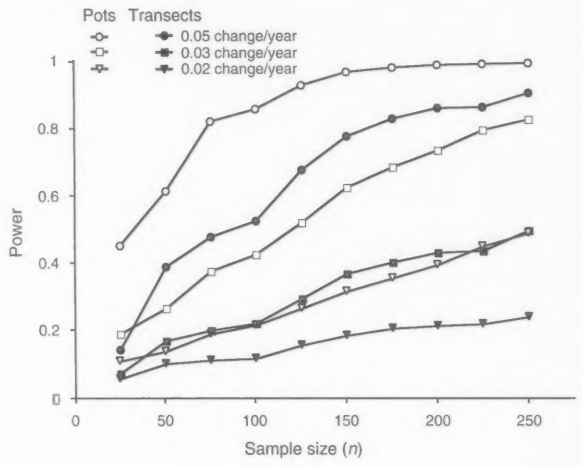

Figure 5

Relationship between power and sample size (n) in comparing catch from pots and density on dive transects for male Dungeness crabs (*C. magister*) at three levels of population change.

In both April and September, pot sampling was significantly biased against females (Fig. 3A). When females were categorized as ovigerous and nonovigerous, it was clear that ovigerous females accounted for the majority of the bias because pots were not biased against nonovigerous females (Fig. 3B). Similar results have been found for a closely related species, *Cancer pagurus*; female *C. pagurus* readily enter pots when they are in a nonovigerous reproductive state but are rarely captured when they are ovigerous (Bennett, 1995). Movement studies of Dungeness crabs tagged with sonic transmitters have demonstrated that ovigerous females move less frequently and move slower than males or nonovigerous females (O'Clair et al., 1990). Thus, one explanation for the bias against ovigerous female crabs is that their restricted movements make it less likely they will be able to locate and become entrapped in pots. In addition to being less mobile, ovigerous females may be less attracted to bait than nonovigerous crabs. In controlled feeding experiments, ovigerous females had lower feeding rates than nonovigerous females, and ovigerous females took longer to begin feeding (Schultz et al., 1996; Schultz and Shirley, 1997). Therefore, ovigerous females may be less responsive to the bait plume from a pot.

The estimate of nonovigerous females from both pot CPUE and dive transect density increased from April to September (Fig. 2, C and D). As with males, the increase in CPUE for nonovigerous females may be partly due to an increase in catchability in September. However, the fact that the density estimates from dives also increased suggests that the number of nonovigerous females actually increased between April and September. This explanation is supported by the decrease in ovigerous crabs from April to September for both CPUE (Fig. 2E) and density estimates (Fig. 2F).

The low catchability of ovigerous females makes it problematic to monitor relative abundance of females or changes in sex ratio through time. However, because pots were not biased against nonovigerous females (Fig. 3), the solution may be to estimate the relative abundance of females by sampling after females hatch their eggs and before they extrude a new clutch of eggs in the fall. In southeastern Alaska, most females are nonovigerous in late July and early August (Stone and O'Clair, 2001; Swiney et al., 2003); therefore this would be the optimal time of year to sample females or to measure sex ratio of Dungeness crab populations. Unfortunately, this timing coincides with the summer commercial fishing season, which could bias sampling if there was "competition" between survey pots and commercial pots.

For both males and females, the power analyses of the pot and dive data indicated that for most population assessment applications it would be extremely difficult to conduct enough dive transects to obtain sufficient statistical power. Even if it were possible to conduct as many dive transects as pot samples, the power of a dive transect was still lower than that of a pot; the higher power of the pots was due to lower variance among pots. Pots work by attracting crabs with a bait plume; thus the area and number of crabs sampled is

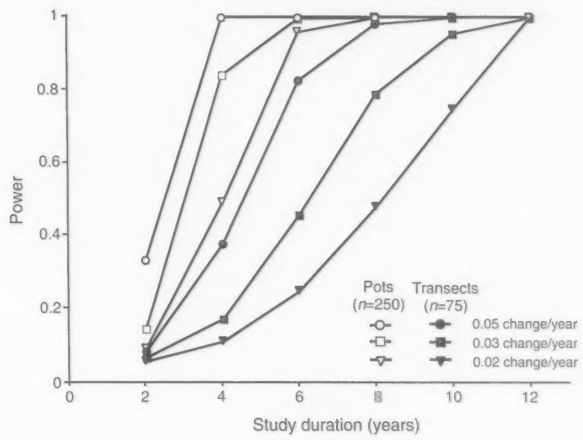

Figure 6

Relationship between power and study duration in comparing catch from crab pots and density on dive transects for male Dungeness crabs (*C. magister*) at three levels of population change. To hold effort constant, we set the sample size (n) to the number of pots and dives that could be accomplished in five days.

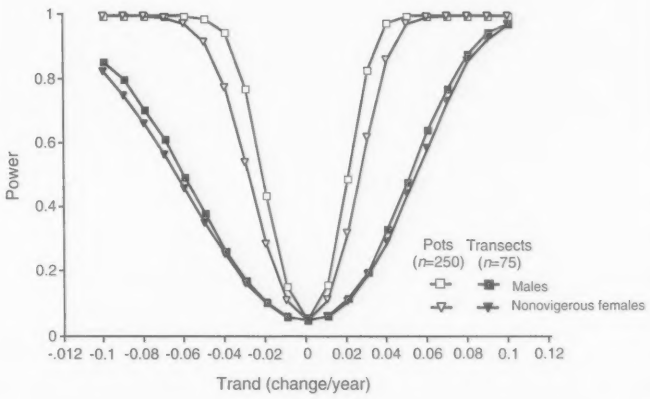

Figure 7

Relationship between power and trend in population in comparing catch in crab pots and density on dive transects for male and nonovigerous female Dungeness crabs (*C. magister*).

larger with pots than with transects and the variance with pots is lower.

Despite their low power, the independent measures of abundance provided by dives helped us identify bias in our Dungeness crab survey method. Our analysis of these two techniques demonstrates that it is possible to avoid most biases with pots if sampling is conducted at optimal times of year. Similar comparisons could be conducted in other areas to identify sampling biases so that they could be minimized and important parameters, such as abundance, size, and sex ratio, could be monitored effectively.

Acknowledgments

This long-term study was made possible by the support of a large number of people. J. de La Bruere made the field work efficient and enjoyable through his expert ability to operate the RV *Alaskan Gyre*. We thank A. Andrews for large efforts during the field work, data management, and analysis. G. Bishop, C. Dezan, E. Hooge, P. Hooge, E. Leder, J. Luthy, J. Nielsen, C. Schroth, D. Schultz, L. Solomon, and K. Swiney each participated in the project for several years. The manuscript was improved by comments from E. Mathews, E. Knudsen, and three anonymous reviewers. We thank M. Jensen, J. Brady, T. Lee, M. Moss, and S. Rice for their continued support. We especially thank the large number of unnamed graduate students, faculty, state and federal agency researchers—over 70 people total—who generously donated their time and efforts to this long-term project. This project was funded by the United States Geological Survey and the National Park Service.

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A method to improve size estimates of walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius*) consumed by pinnipeds: digestion correction factors applied to bones and otoliths recovered in scats

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Abstract—The lengths of otoliths and other skeletal structures recovered from the scats of pinnipeds, such as Steller sea lions (*Eumetopias jubatus*), correlate with body size and can be used to estimate the length of prey consumed. Unfortunately, otoliths are often found in too few scats or are too digested to usefully estimate prey size. Alternative diagnostic bones are frequently recovered, but few bone-size to prey-size correlations exist and bones are also reduced in size by various degrees owing to digestion. To prevent underestimates in prey sizes consumed techniques are required to account for the degree of digestion of alternative bones prior to estimating prey size. We developed a method (using defined criteria and photo-reference material) to assign the degree of digestion for key cranial structures of two prey species: walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius*). The method grades each structure into one of three condition categories; good, fair or poor. We also conducted feeding trials with captive Steller sea lions, feeding both fish species to determine the extent of erosion of each structure and to derive condition-specific digestion correction factors to reconstruct the original sizes of the structures consumed. In general, larger structures were relatively more digested than smaller ones. Mean size reduction varied between different types of structures (3.3–26.3%), but was not influenced by the size of the prey consumed. Results from the observations and experiments were combined to be able to reconstruct the size of prey consumed by sea lions and other pinnipeds. The proposed method has four steps: 1) measure the recovered structures and grade the extent of digestion by using defined criteria and photo-reference collection; 2) exclude structures graded in poor condition; 3) multiply measurements of structures in good and fair condition by their appropriate digestion correction factors to derive their original size; and 4) calculate the size of prey from allometric regressions relating corrected structure measurements to body lengths. This technique can be readily applied to piscivore dietary studies that use hard remains of fish.

Manuscript submitted 28 April 2003 to Scientific Editor's Office.

Manuscript approved for publication 25 March 2004 by the Scientific Editor.

Fish. Bull. 102:498–508 (2004).

Prey skeletal remnants from stomach samples and more recently from fecal (scat) samples are widely used to determine what pinnipeds eat (Pitcher, 1981; Olesiuk et al., 1990; Tollit and Thompson, 1996; Browne et al., 2002). Prey can usually be identified from taxon-specific hard remains, the sizes of which often correlate with the length and mass of the prey (Härkönen, 1986; Desse and Desse-Berset, 1996). In the past, sagittal otoliths were commonly used to estimate prey size (Frost and Lowry, 1981) but were recognized to erode or become completely digested (Prime and Hammond, 1987; Harvey, 1989). Thus, otolith measurements likely underestimated sizes and numbers of fish ingested (Jobling and Breiby, 1986), thereby preventing a reliable assessment of overlap of prey consumed with catch taken by commercial fisheries (Beverton, 1985). Accurate estimates of size of prey consumed by pinnipeds are also important in order to understand foraging behavior and to explain spatial and temporal variability in diet composition.

There are at least three potential ways to deal with the effect of digestion on estimates of prey size. One is to measure only relatively uneroded otoliths and assume that eroded otoliths are from the same size fish as uneroded otoliths (Frost and Lowry, 1986; Bowen and Harrison, 1994). Another is to apply a single species-specific digestion coefficient or correction factor (DCF), derived from feeding experiments with captive seals fed fish of known sizes and using measurements of all the eroded otoliths recovered in the scats produced (Prime and Hammond, 1987; Harvey, 1989). The third is to estimate and correct for the degree of digestion (based on defined losses of morphological features) of each recovered otolith by using estimates from reference material (Sinclair et al., 1994; Antonelis et al., 1997) or by applying condition-specific DCFs derived from fish fed in captive seal feeding studies (Tollit et al., 1997).

Of the three approaches to correctly estimate prey size from skeletal remains, there is the assumption with

the use of only uneroded otoliths that recovery and the degree of digestion is independent of otolith size, resulting in a potentially biased fraction. For certain species it can also result in a notable reduction in sample size because relatively few otoliths pass through the gut in good condition. The second approach of applying mean species-specific DCFs is an improvement to not accounting for size reduction (Laake et al., 2002); however, there is the assumption with this approach that all structures are reduced in size by the same amount. Consequently, mean fish mass may be overestimated if such correction factors are applied to relatively undigested otoliths, or they may be underestimated if applied to very digested otoliths (Hammond et al., 1994; Tollit et al., 1997). The third method accounts for the intraspecific variation in size reduction caused by digestion, reduces systematic error (see Hammond and Rothery, 1996), yields estimates of mass that compare favorably to those fed to captive animals (Tollit et al., 1997), and hence may well be the most promising approach to reconstructing prey size.

The dramatic decline of the western population of Steller sea lions (*Eumetopias jubatus)* in the 1980s (Loughlin et al., 1992; Trites and Larkin, 1996) has prompted a number of studies to determine what they eat and the extent of dietary overlap (prey consumed) with catch taken by commercial fisheries. Stomach contents analysis was used to determine diet until the late 1980s when scat analysis became the preferred method (e.g., Pitcher, 1981; Frost and Lowry, 1986; Sinclair and Zeppelin, 2002). However, unlike in stomachs, there is an overall sparsity of otoliths in Steller sea lion scats (Sinclair and Zeppelin, 2002) and, therefore there is a need to also use other skeletal structures to describe the size of prey consumed.

The following outlines a method (using defined criteria and photo-reference material) to assign the degree of digestion for otoliths and alternative key skeletal structures of walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius)* recovered from scats. We also present the results of a feeding study with captive Steller sea lions used to determine the extent of erosion and to derive condition-specific digestion correction factors to reconstruct the original sizes of the pollock and Atka mackerel structures consumed. Finally, we combine these DCFs with newly developed regression formulae that estimate fish length to derive a more accurate method of estimating size of pollock and Atka mackerel consumed by Steller sea lions and other pinnipeds (see Zeppelin et al., 2004, this issue; Tollit et al., 2004, this issue).

Materials and methods

Experimentally derived digestion correction factors

Feeding experiments were conducted with two 3-year-old female Steller sea lions: Steller sea lion 1 [SSL1] [ID no. F97HA], mean mass 129 kg; steller sea lion 2 [SSL2] [ID no. F97SI], mean mass 150 kg) between October 2000 and April 2002 at the Vancouver Aquarium Marine Science Centre. Over the experimental period, the sea lions were fed pollock for 52 days in 16 separate feeding experiments, and Atka mackerel for 31 days in 5 separate feeding experiments, at between ~4–8% of body mass per day. Fork length (FL) and weight of all fish were measured to ±0.1 cm and ±1 g. Sea lions were fed meals of pollock of three size categories (small, 28.5–32.5 cm FL; medium, 33.5–38.7 cm FL; large, 40–45 cm FL) and meals of Atka mackerel of one size category (30–36 cm FL). Fish of one particular size category were fed either as a single meal or as a seven-day block of meals. Full details of a typical experimental protocol can be found in Tollit et al. (2003). Size ranges for any category of fish fed within separate experiments were usually ≤3 cm. Fecal material was collected until no other remains of experimental meals were found (7 days after feeding), and was washed through a 0.5-mm sieve to remove hard parts. Each animal was maintained on whole Pacific herring (*Clupea pallasi*) between experiments at ~6% body mass per day.

The strong relationship between fish size and otolith size also exists for other skeletal structures (Desse and Desse-Berset, 1996). Thus, we quantified the types and numbers of the prey structures recovered in the scats of free-ranging Steller sea lions (from the collections of Trites et al.[1] and Sinclair and Zeppelin, 2000) and selected seven of the most commonly occurring structures for pollock and Atka mackerel. These were the sagittal otolith (OTO), as well as the interhyal (INTE), hypobranchial 3 (HYPO), pharyngobranchial 2 (PHAR), angular (ANGU), quadrate (QUAD), and the dentary (DENT). The structures selected also had particular morphological features that seemed to be relatively resistant to digestion and could effectively be used to estimate fish size (Figs. 1 and 2, Table 1).

Concurrent with our feeding study, we measured selected structures (Figs. 1 and 2) from randomly subsampled fresh fish and combined these data with unpublished NMFS data to generate allometric regression formulae relating structural measurements to fish length (see Zeppelin et al., this issue). Fork lengths (±0.1 cm) and weights (±1 g) of an extended subsample of pollock (8.3–47.7 cm FL) were measured to generate an appropriate regression formula for estimating fish mass from fork length estimates. All selected structures are located in the cranium as illustrated in Zeppelin et al. (2004, this issue). Naming of fish structures follows Rojo (1991).

Initial inspection of selected structures found in scats from the wild revealed high intraspecific variation in the degree of digestion, ranging from no apparent size reduction to about a 60% size reduction (heavily digested material). Consequently, we extended the condition-

[1] Trites, A. W., D. G. Calkins, and A. J. Winship. 2003. Unpubl. data. Marine Mammal Research Unit, Fisheries Centre, University of British Columbia, Hut B-3, 6248 Biological Sciences Road, Vancouver, B.C., Canada, V6T 1Z4.

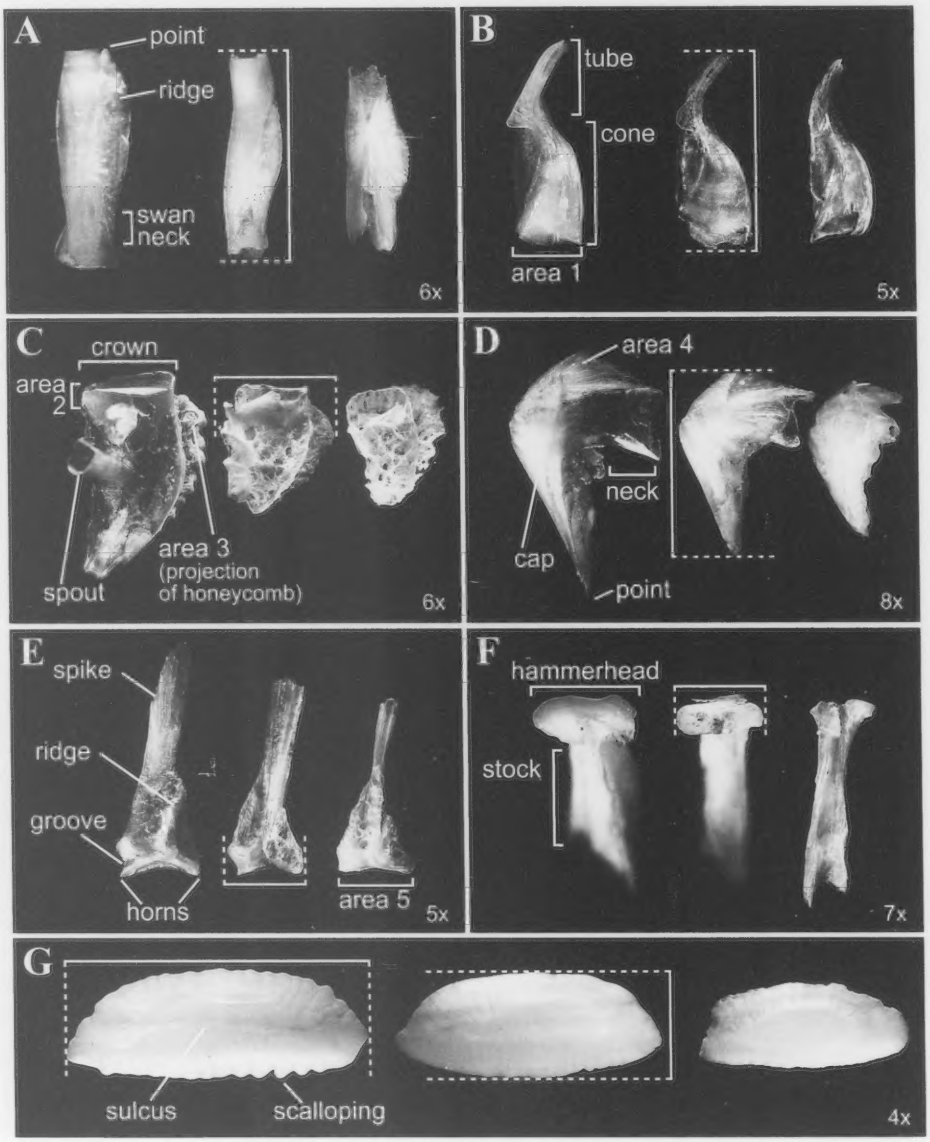

Figure 1

Photographs showing the changes in morphological features in seven cranial structures of walleye pollock (*Theragra chalcogramma*) resulting from digestion. Within each section of the figure three condition categories (good, fair, and poor) are represented from left to right for (**A**) interhyal (INTE), (**B**) hypobranchial 3 (HYPO), (**C**) pharyngobranchial 2 (PHAR), (**D**) angular (ANGU), (**E**) quadrate (QUAD), (**F**) dentary (DENT) and (**G**) sagittal otolith (OTO). Key features used in classification are labeled (see Table 1 for details), and the measurements taken to calculate fish length (solid line between dashed lines).

specific DCF technique described by Tollit et al. (1997). We began by examining the external morphological features and surface topography of selected structures from undigested fish (<12 cm to >53 cm) and compared these with the topography of the same structures recovered from scats collected from wild and captive animals

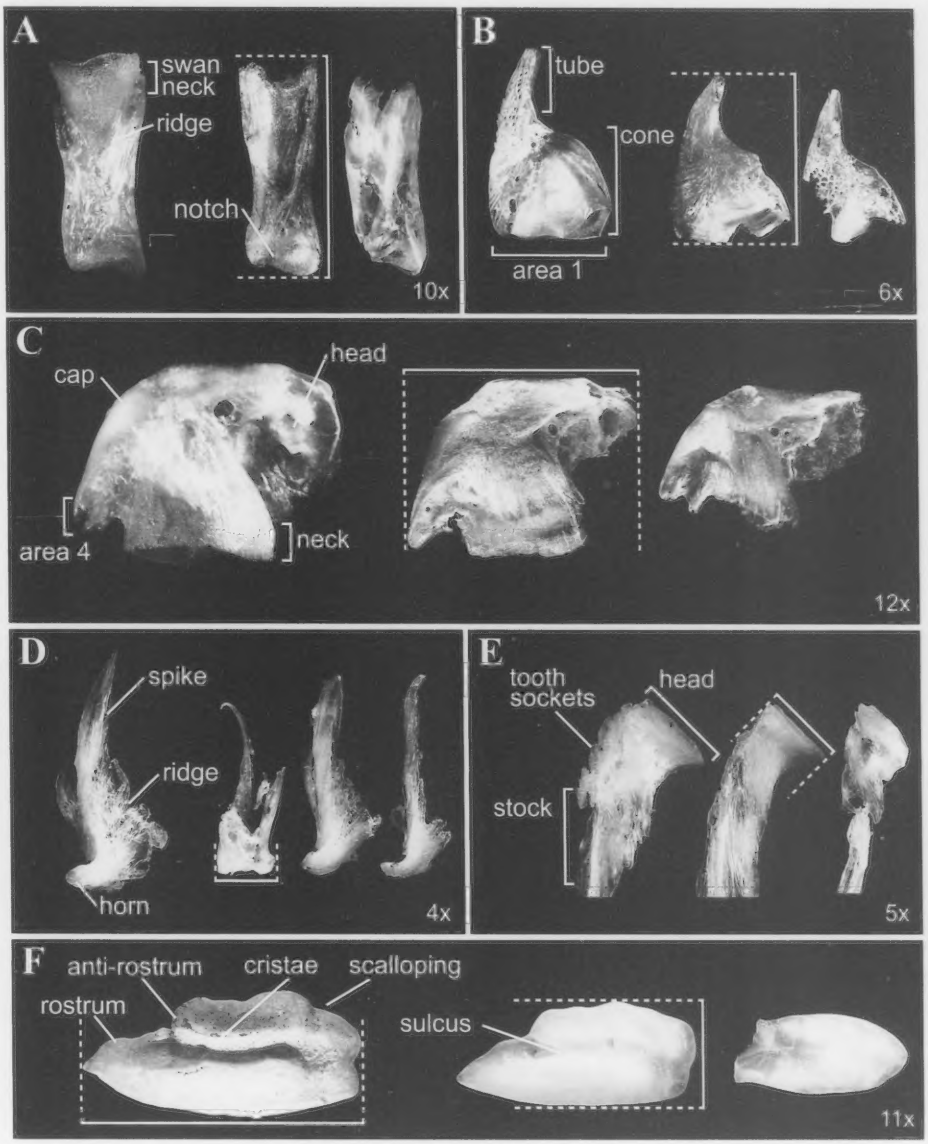

Figure 2

Photographs showing the changes in morphological features in seven cranial structures of Atka mackerel (*Pleurogrammus monopterygius*) resulting from digestion. Within each section of the figure three condition categories (good, fair, and poor) are represented from left to right for (**A**) interhyal (INTE), (**B**) hypobranchial 3 (HYPO), (**C**) angular (ANGU), (**D**) quadrate (QUAD), (**E**) dentary (DENT) and (**F**) sagittal otolith (OTO). Key features used in classification are labeled (see Table 1 for details) and measurements taken to calculate fish length (solid line between dashed lines).

(Figs. 1 and 2). The morphological features used to assess level of digestion showed no differences in relative shape, structure, or in proportion across the size range of fresh fish examined. We then devised a criteria-based method to assign a condition category to each structure depending on the degree of digestion. These criteria

take into account only the loss of size to the relevant feature being measured to estimate fish length (Figs. 1 and 2).

The grading criteria for otoliths (OTO) were based on the condition categories developed by Sinclair et al. (1996) to investigate prey selection by northern fur seals (*Callorhinus ursinus*). As seen in Sinclair et al. (1996) and other studies (Frost and Lowry, 1986; Tollit et al., 1997), external features such as lobation and the general shape and definition of the sulcus were found in our study to be good indicators of the degree of otolith digestion. For the remaining cranial bones, digestion indicators included the loss of definition or breakage of defined structural features such as the horns and ridge (QUAD), hammerhead and stock (DENT), swan neck, notch and ridge (INTE), honeycomb and crown (PHAR), cap, neck, and head (ANGU) and tube and cone (HYPO). We used changes in the described condition-category criteria (see Table 1 for full details) in tandem with photo-reference material (Figs. 1 and 2) to classify all structures into one of three digestion grades or condition categories: "good", "fair," or "poor."

Hard parts recovered from feeding experiments were sorted, and all selected cranial structures were assigned a condition category and measured with calipers to within ±0.01 mm. Because otoliths were often chipped or partly broken lengthwise, both length and width were measured. To test our grading technique, an independent observer (T.Z.) reassigned a random subsample of each condition category of pollock structures (n=158) in a blind test.

On initial investigation, high intraspecific variation was observed within the selected structures assigned in poor condition in our feeding study with captive Steller sea lions. Consequently, structures in poor condition were not used to calculate DCFs for this category. Our basis for exclusion was supported by the work of Sinclair et al. (1994) and Tollit et al. (1997). Captive sea lions in our study occasionally regurgitated prey in the swim tank. Recovered structures that we considered to have been regurgitated were excluded from DCF calculations (i.e., vertebrae still articulated, bones that had flesh attached or that were of a size to exclude passage through the pyloric sphincter).

Mean reduction (MR) in the metric of each structure (s) recovered from our feeding experiment was estimated for each remaining condition category (c) according to

$$MR_{s,c} = \left(1 - \frac{\bar{E}_{s,c}}{\bar{I}_s}\right) \times 100,$$

where the mean size of egested structures ($\bar{E}$) of each condition category was calculated from measurements of those recovered from the captive feeding experiments, and the mean size of each ingested structure ($\bar{I}_s$) was estimated from the fork length of fish fed by using inverse predictions of the regression formulae derived from fresh material (Zeppelin et al., 2004, this issue). Mean ingested size was estimated by using bootstrap simulations (1000 runs) that randomly sampled with replacement and selected the median (500th value) from the sorted bootstrapped values (Reynolds and Aebischer, 1991).

For pollock, mean reduction for each condition category was compared across size ranges by using a Kruskal-Wallis analysis of variance. A significance level of P<0.0056 was set based on the Bonferroni adjusted probability for nine multiple comparisons (Siegel and Castellan, 1988). Failing to find any significant differences resulted in pooling the data from each size range to calculate specific condition category MR values. Condition category DCFs were calculated for each selected structure as $\bar{I}_s/\bar{E}_{s,c}$ except for PHAR structures of Atka mackerel because too few elements were recovered from the scats of captive animals.

Estimating confidence limits around digestion correction factors

We used a bootstrap simulation to estimate upper and lower bounds of the 95% confidence interval (CI) given that the DCF is a ratio of two means (Reynolds and Aebischer, 1991). This technique allows different sources of error to be combined or partitioned. There were two major sources of error associated with calculating DCFs (Tollit et al., 1997). The first were those associated with the regression formulae used to calculate the mean size of structure ingested from the original fish fed, and the second were those associated with the errors around the mean size of egested structure (i.e., resampling errors).

We assessed errors associated with the regression formulae using a parametric bootstrapping procedure (Manly, 1997) that involved regressing structure size against fork length. This was repeated 1000 times and 95% confidence intervals were taken as the 25th and 975th values of the sorted bootstrapped regression coefficient values. Results were compared to those computed analytically by using the resultant standard error (Eq. 17.23 in Zar, 1984) and were found to be consistent (see Zeppelin et al., 2004, this issue).

We estimated resampling errors related to the variability in digestion of egested structures by repeatedly selecting n structures, at random, with replacement from the original sample set of n egested structures. Mean egested size was recalculated in this way 1000 times, as were a mean DCF and 95% CI as described above. Both regression and resampling errors were combined in sequence to derive overall 95% CIs around DCFs.

Our recommended procedure for applying our DCFs to cranial structures recovered from scats collected in the wild has four steps: 1) measure the recovered structures and grade the extent of digestion using defined criteria and photo-reference collection; 2) exclude structures graded in poor condition; 3) multiply measurements of structures in good and fair condition by their appropriate digestion correction factors to derive their original size; and 4) calculate the size of prey from allometric regressions relating corrected structure measurements to fish fork lengths (see also Tollit et al., 2004, this issue).

Results

A relatively objective method to estimate the degree of digestion of dominant structures of pollock and Atka mackerel was derived by using defined criteria (Table 1) and photo-reference material (Figs. 1 and 2). Condition-specific digestion correction factors (and derived confidence intervals) calculated for each structure augmented our method of estimating size of prey from bones and otoliths recovered in scats, as well as potentially from bones and otoliths taken from stomach contents.

Mean reduction (MR) in the size of pollock DENT and QUAD in good condition and ANGU, HYPO, INTE, OTO, and PHAR in fair condition were between 12.2–18.5%, and larger values were found for QUAD (22.8%) and DENT (24.7%) in fair condition (Table 2). Our overall 95% confidence intervals were generally symmetrical and converted to a mean range of ±2.2% (±0.5, SD) around MR values. Mean DCFs ranged between 1.14 and 1.33, and lower bounds of 95% CIs exceeded 1.11 in all instances, confirming that egested structures of these condition categories were significantly smaller than the size at which they were ingested (Table 2). Partitioning errors showed that resampling of egested structures was the major source of error (>73% across structures) within the overall total. Our overall 95% CIs resulted in a maximum total error of ±1.7 cm around an estimated mean of 40 cm for pollock.

Mean reduction in the size of Atka mackerel structures varied more widely (3.3–26.3%), leading to DCFs ranging between 1.03 and 1.36. QUAD in good condition provided the smallest DCF, and DENT in fair condition the largest. Overall, our 95% CIs converted to a mean range of ±2.4% (±0.6) around MR values, and all lower 95% CI bounds exceeded 1.0 (Table 2). As seen when errors were partitioned for pollock, errors owing to resampling of egested structures were the major source of error (>83% across structures) within the overall total for Atka mackerel. Our overall 95% CIs resulted in a maximum total error of ±1.2 cm around an estimated mean of 40 cm for Atka mackerel.

With the exception of the two largest skeletal structures (DENT and QUAD, Table 2), some selected structures (INTE, HYPO, PHAR, ANGU, and OTO) occurred in scats with no clear loss in size or loss of morphological features related to digestion. For these five structures, we ascribed the condition category good and assigned a DCF of 1.0 (i.e., no correction for partial size reduction due to digestion required).

Of the 158 structures in our blind test, 141 (89.2%) were assigned identical condition categories. Of the remaining 17 structures, 11 (65%) were noted as being borderline between categories. Angulars (ANGU) accounted for the majority (~60%) of all differences, with all but one re-assigned in good condition as opposed to fair condition. On review, differences in assigning angulars were mainly the result of differences in opinion on what constituted a well-defined and sharp point (Fig. 1, Table 1). Clarification through the additional use of reference material (including both pristine structures and examples of each condition category) is advised, particularly for angulars. Comparison of the same 158 bones between two observers (D.T. and S.H.) using the same structure reference collection resulted in assigning more than 93% (147/158) of structures to an identical category.

The regression formula for estimating pollock mass (M) from fork length (FL) estimates was best described by using an exponential equation ($M=0.0051\times FL^{3.11}$, $n=981$, $r^2=0.987$).

Discussion

The size of prey consumed by pinnipeds can usually be reliably estimated from otoliths recovered in scats if partial digestion is accounted for (Tollit et al., 1997). However, otoliths from Steller sea lion scats are often found in too few numbers, or are too digested or broken to be useful (Sinclair and Zeppelin, 2002; Tollit et al., 2004, this issue). It was, therefore, necessary to use alternative skeletal structures to estimate the size of prey selected by Steller sea lions. Zeppelin et al. (2004, this issue) documented good relationships ($r^2=0.78–0.99$) between the size of selected alternative structures and fork length for pollock and Atka mackerel. However, all skeletal structures are susceptible to digestion in the stomach (our study, and Murie and Lavigne, 1986). Thus, techniques are required to account for the degree of digestion of alternative structures prior to estimating prey size.

Reductions in the size of otoliths during passage through the digestive tract of pinnipeds have been widely reported (e.g., da Silva and Neilson, 1985; Prime and Hammond, 1987; Harvey, 1989; Tollit et al., 1997). Similarly, we found significant reduction in the sizes of all selected cranial structures from pollock and Atka mackerel. Size reduction also showed great variability. Relatively small structures were found with no obvious loss in size due to digestion, but were also frequently heavily eroded.

The degree of digestion on different otoliths and bones may be related to species, size of fish (Bowen, 2000), or even its shape, but seems to be random in any one meal (Murie and Lavigne, 1986). Degree of digestion likely depends on a range of factors such as meal size, meal frequency, meal composition, and method of consumption. In the face of these multiple factors we feel our method for classifying the degree of digestion into one of three condition categories is practical and relatively objective. However, our technique does not consider potential biases of enumeration associated with smaller prey being more susceptible to complete digestion than relatively larger prey, or of individual fish being counted more than once if all multiple structures are used. Nevertheless, resolution to these biases have been advocated (see Tollit et al., 1997; Laake et al., 2002; Tollit et al., 2003; Tollit et al., 2004, this issue). The category selections chosen with our criteria showed good agreement among independent observers.

Table 1

Distinctive external morphological features for defining the degree of digestion (condition category) as good (G), fair (F), and poor (P) for selected cranial structures of walleye pollock and Atka mackerel. Features are given in order of importance. See Table 2 for definition of structure codes and Figure 1 and 2 for illustrations. WP = walleye pollock.

Species and structure code	Category	Distinctive external morphological features
Walleye pollock		
INTE	G	1) Retains characteristic shape, notably the ridge and swan neck. 2) Both ends show no damage (except for the loss of the point and minor nicks) and do not affect length measurement.
	F	1) Ridge and swan neck clearly defined. 2) One end can show limited damage with <15% reduction. Minor nicks on opposite end acceptable, if there is no further loss in length measurement.
	P	1) Loss of characteristic shape, with ridge or swan neck (or both) ill defined. Body of structure contains holes. 2) Both ends show clear damage.
HYPO	G	1) Retains characteristic shape, with cone ~2× the length of the tube. 2) Tube end and area 1 show no damage (except for minor nicks) and do no affect the total length measurement. 3) Cone end angled when viewed from the front elevation (back elevation shown in Fig. 1).
	F	1) Tube end or area 1 shows limited damage (cone end no longer angled) clearly preventing an accurate length measurement.
	P	1) Both tube end and area 1 show damage, and a general loss of characteristic shape evident.
PHAR	G	1) Retains characteristic shape, notably a raised spout, honeycomb, and crown. 2) Crown clearly projects above honeycomb (front elevation) and is intact at area 2. 3) Clear projection of honeycomb (back elevation—see area 3). 4) No affect on measurement.
	F	1) No clear projection of honeycomb at area 3 or crown shows damage at area 2 (preventing an accurate width measurement). 2) Crown or spout (or both) can show minor damage.
	P	1) Characteristic shape lost, often only honeycomb present. 2) Honeycomb smooth, crown heavily eroded with areas 2 and 3 eroded or damaged. 3) Both ends show clear damage.
ANGU	G	1) Point sharp and well defined with no impact to measurement. 2) Area 4 in good condition and angled curve complete. 3) Neck present, but with minor damage. 4) Material of cap continues to point tip.
	F	1) Point no longer extensive or sharp or area 4 damaged and poorly defined. 2) Neck usually present, but with wear.
	P	1) Characteristic shape lost with neck often absent. 2) Point heavily eroded. 3) Area 4 shows damage or no definition.
QUAD	G	1) Groove defined from all angles and observable with the naked eye. 2) Horns rounded. 3) Angle of area 5 is clearly curvilinear. 4) Evidence of ridge and spike often observable.
	F	1) Groove unclear, forming only an indisctinct notch. 2) Horns have lost rounded definition and may be pointed or worn on one side. 3) Ridge and spike often only residual.
	P	1) Horns pointed, notch absent. 2) Ridge and spike often absent. 3) Angle of area 5 flattened. 4) Unable to determine side with assurance.
DENT	G	1) Hammerhead retains rounded end elevation features (note: both sides are not exactly symmetrical), allowing full width measurement. 2) Material in addition to the stock may be present. 3) Stock clearly curved from side elevation. 4) Width and breadth of "rounded" stock similar.
	F	1) Hammerhead shows erosion on one side, affecting full width measurement. 2) Breadth of stock reduced, but not flattened.
	P	1) Hammerhead eroded and flattened with both sides showing erosion. 2) Breadth of stock flattened, stock less rounded and less robust. 3) Unable to determine side with assurance.
OTO	P	1) Sulcus and scalloping (on most margins) well defined, and no obvious reduction in size due to digestion. 2) Able to determine side. 3) Inside strongly convex, retains characteristic shape.
	F	1) Sulcus worn but shows definition. 2) Able to determine side. 3) Scalloping worn but shows no reverse scalloping.
	P	1) Unable to determine side. 2) Scalloping worn completely smooth and reverse scalloping present. 3) Clearly broken, worn, flattened, and unable to obtain an accurate measurement.

continued

Table 1 (continued)

Species and structure code	Category	Distinctive external morphological features
Atka mackerel		
INTE	G	Like that of walleye pollock (WP) (except no point), with ridge, neck and notch clearly defined.
	F	1) Ridge present, but shows signs of wear. 2) Swan neck shows wear resulting in a "horseshoe" shape. 3) Notch shows only minor wear or chipping and does not prevent accurate measurement.
	P	1) Loss of characteristic ridge and neck with body worn (may contain holes). 2) Both neck and notch show clear damage.
HYPO	G	1) Cone rounded and complete, tube complete, retains characteristic shape. 2) Minor nicks on cone and tube may be present but do not impact total length measurement.
	F	Cone worn, loss of rounded shape, and area 1 shows minor chipping or damage or tip of tube is broken or clearly chipped.
	P	1) Cone body and area 1 show major wear, chips, and breaks. 2) Tube broken or absent entirely, unable to measure length.
ANGU	G	Like WP, additionally cap rounded and head shows only minor wear.
	F	Like WP, additionally 1) cap worn with loss of shape, 2) Head worn, chipped, and often has holes, 3) Ridge on dorsal side above neck worn smooth.
	P	Like WP, additionally 1) head shows major damage, wear, breaks, and holes, 2) Difficult to determine side with confidence.
QUAD	G	1) Horns rounded and in good condition, with angle between horns clearly curvilinear. (Note: Horns are of unequal size and shape and one side is more robust, rounded, and sloped.) 2) Evidence of ridge and spike observable. 3) Definition of left and right sides is easily achievable.
	F	1) Horns have lost rounded definition and may be pointed or worn on one side, making distinction between sides difficult. 2) Ridge and spike often only residual.
	P	Like WP, additionally no distinction between horns easily achievable.
DENT	G	Like WP (except no hammerhead), additionally 1) head retains characteristic features, tooth sockets present, 2) Ventral side of head a defined point.
	F	Like WP, additionally 1) head eroded or chipped with tooth sockets noticeably worn, 2) Point on ventral side of head eroded or chipped.
	P	Like WP, additionally head eroded or flattened with point often heavily eroded or badly chipped, accurate measurement unattainable.
OTO	G	1) Rostrum not chipped or broken. 2) Sulcus clearly defined, as are anterior and posterior colliculums. 3) Scalloping on antirostrum and posterior end clearly distinguishable. 4) No obvious wear or chipping with no obvious reduction in length (width). 5) Cristae of antirostrum forms a well-defined ridge.
	F	1) Rostrum shows some wear but remains unbroken and retains characteristic shape. 2) Sulcus still has definition despite wear, shown as a uniform channel, anterior and posterior colliculums indistinct. 3) Cristae and scalloping on antirostrum and posterior end worn smooth.
	P	1) Rostrum or posterior end broken or worn to such a degree that accurate measurement cannot be obtained. 2) Sulcus difficult to distinguish or worn smooth. 3) Cristae and scalloping on antirostrum and posterior end worn completely smooth. 4) Side cannot be easily obtained.

Nevertheless, we recommend that a hands-on reference collection be used.

The procedure we recommend to estimate fish length after classification involves excluding structures considered heavily digested (condition category poor) and applying specific condition-category DCFs (Table 2) to the remaining structure prior to calculating fish length from allometric regressions (see Tollit et al., 2004, this issue). The exclusion of structures in poor condition was necessary because of the large and variable size reduction observed in this category. Our technique uses changes noted in the morphological features of the structures themselves and is therefore not specific to Steller sea lions. Because structures are likely to erode in a predictable manner whatever the species of the stomach they are held within, it seems probable that they can also be classified into a particular condition category for use with DCFs. Consequently, our technique may be appropriate to marine piscivore dietary studies where prey size needs

Table 2

Condition-specific digestion correction factors (DCFs) for selected cranial structures of walleye pollock and Atka mackerel with associated condition categories good (G) and fair (F). Lower and upper bounds of the 95% confidence intervals (CIs) were calculated by using bootstrap resampling procedures.

Species and structure	Structure code	Grade	*n*	DCF	SD	CI	
						Lower	Upper
Walleye pollock							
Interhyal	INTE	F	54	1.1423	0.054	1.1168	1.1714
Hypobranchial 3	HYPO	F	22	1.1658	0.063	1.1343	1.1970
Pharyngobranchial 2	PHAR	F	39	1.2109	0.067	1.1717	1.2566
Angular	ANGU	F	85	1.2065	0.103	1.1670	1.2462
Quadrate	QUAD	G	20	1.2272	0.039	1.2025	1.2512
		F	27	1.2958	0.074	1.2623	1.3280
Dentary	DENT	G	17	1.1950	0.074	1.1546	1.2337
		F	31	1.3285	0.071	1.2941	1.3649
Otolith (length)	OTOL	F	37	1.1593	0.059	1.1400	1.1788
Otolith (width)	OTOW	F	49	1.2107	0.089	1.1901	1.2419
Atka mackerel							
Interhyal	INTE	F	37	1.0729	0.089	1.0374	1.1085
Hypobranchial 3	HYPO	F	23	1.1361	0.040	1.1160	1.1568
Angular	ANGU	F	40	1.1361	0.097	1.1053	1.1700
Quadrate	QUAD	G	23	1.0343	0.053	1.0070	1.0597
		F	23	1.0886	0.078	1.0551	1.1213
Dentary	DENT	G	34	1.2068	0.098	1.1666	1.2466
		F	37	1.3563	0.143	1.3063	1.4119
Otolith (length)	OTOL	F	109	1.1691	0.109	1.1459	1.1921
Otolith (width)	OTOW	F	115	1.2062	0.104	1.1837	1.2277

to be determined from partially digested prey hard remains.

Experimentally derived pollock DCFs were determined from three distinct size ranges of fish (28.5–45 cm FL), but the degree of erosion for each structure within each condition category did not show any significant differences across this range. We also found the relative shape, structure, and proportion of the morphological features used to estimate erosion were consistent for both smaller and larger fish. We therefore believe DCFs can be used for fish outside of the experimental size range of this study. Average size reduction varied between different pollock structures (12.2–24.7%) and also between condition categories, as they did for Atka mackerel (Table 2). We determined that pollock otoliths in fair condition were reduced by 14% in length, close to the 20% value estimated from reference material (Sinclair et al., 1994). Our criteria for defining a condition category of fair for pollock otoliths equates to a grade between low amounts and medium amounts of digestion as defined by Tollit et al. (1997) for Atlantic cod (which has a similar looking otolith). Our value of 14% lies midway between those determined for cod otoliths graded low and medium.

Jaw bones (DENT) were by far the largest structure used in our study but do not appear to pass through the pyloric sphincter without some level of digestion. Usually only the hammerhead and stock (representing less than a third of the whole structure) are recovered in scats. The large size accounts for the relatively greater percent mean reduction and hence higher DCF of DENT structures graded either in good or fair condition (Table 2). Although quadrates (QUAD) are also relatively large structures with a projecting ridge that is often much reduced when found in scats, we found QUAD structures of Atka mackerel recovered in scats from field studies and captive sea lion studies in relatively better condition than those of pollock, leading to differences in grading criteria and resulting DCFs (Tables 1 and 2). Part of the reason may be that the horns on a pollock QUAD project widthwise more than those of Atka mackerel, presenting a greater surface area for digestive erosion of the structural feature that is measured to estimate size (Fig. 1).

Our overall 95% confidence intervals around DCFs were generally narrow (Table 2), highlighting the tight fit of the regression formulae used and the benefits of partitioning the data into specific categories. Our bootstrap analysis suggests that resampling errors were the major source of error in calculating DCFs. Future research should concentrate on improving sample sizes for data on percentage size reduction of bones for each

category, rather than on improving regression formulae. For both prey species, QUAD in good condition and OTO in fair condition, in addition to pollock INTE in fair condition and Atka mackerel HYPO in fair condition, provided the most reliable estimates of prey size (Table 2). DENT in fair condition, particularly for Atka mackerel, provided the least reliable estimate of prey size (Table 2). Measurement error was relatively insignificant, but attention should be taken when measuring ANGU and HYPO (Tollit et al., 2004, this issue).

Companion studies by Tollit et al. (2004, this issue) and Zeppelin et al. (2004, this issue) demonstrate the feasibility of applying DCFs to structures other than otoliths and the need to consider the degree of digestion to correctly estimate the length of prey eaten by pinnipeds and other piscivores. Applying appropriate digestion correction factors will lead to more refined estimates of consumption (mass of prey) by marine mammals, as well as the extent of potential overlap (length of prey) with the length of fish caught by commercial fisheries.

Acknowledgments

Funding was provided to the North Pacific Universities Marine Mammal Research Consortium by the National Oceanographic Atmospheric Administration and the North Pacific Marine Science Foundation. We would like to thank the marine mammal trainers and staff of the Vancouver Aquarium Marine Science Centre, the contribution of personnel of the UBC Marine Mammal Research Unit of the UBC EM facility, J. L. Laake for statistical advice, T. J. Orchard, C. J. Gudmundson, S. J. Crockford, M. Wong, E. H. Sinclair, and two anonymous reviewers. We would also like to express gratitude to the organizations and companies that have donated fish to the project. Work was undertaken in accordance with UBC Animal Care Committee guidelines.

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Sizes of walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius*) consumed by the western stock of Steller sea lions (*Eumetopias jubatus*) in Alaska from 1998 to 2000

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Abstract—Prey-size selectivity by Steller sea lions (*Eumetopias jubatus*) is relevant for understanding the foraging behavior of this declining predator, but studies have been problematic because of the absence and erosion of otoliths usually used to estimate fish length. Therefore, we developed regression formulae to estimate fish length from seven diagnostic cranial structures of walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius*). For both species, all structure measurements were related with fork length of prey (r^2 range: 0.78–0.99). Fork length (FL) of walleye pollock and Atka mackerel consumed by Steller sea lions was estimated by applying these regression models to cranial structures recovered from scats (feces) collected between 1998 and 2000 across the range of the Alaskan western stock of Steller sea lions. Experimentally derived digestion correction factors were applied to take into account loss of size due to digestion. Fork lengths of walleye pollock consumed by Steller sea lions ranged from 3.7 to 70.8 cm (mean=39.3 cm, SD=14.3 cm, n=666) and Atka mackerel ranged from 15.3 to 49.6 cm (mean=32.3 cm, SD= 5.9 cm, n=1685). Although sample sizes were limited, a greater proportion of juvenile (≤20 cm) walleye pollock were found in samples collected during the summer (June–September) on haul-out sites (64% juveniles, n=11 scats) than on summer rookeries (9% juveniles, n=132 scats) or winter (February–March) haul-out sites (3% juveniles, n=69 scats). Annual changes in the size of Atka mackerel consumed by Steller sea lions corresponded to changes in the length distribution of Atka mackerel resulting from exceptionally strong year classes. Considerable overlap (>51%) in the size of walleye pollock and Atka mackerel taken by Steller sea lions and the sizes of these species caught by the commercial trawl fishery were demonstrated.

Manuscript submitted 28 April 2003 to Scientific Editor's Office.

Manuscript approved for publication 25 March 2004 by the Scientific Editor.

Fish. Bull. 102:509–521 (2004).

The western stock of Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska and the Bering Sea has experienced dramatic and continued declines since the mid-1970s (Loughlin et al., 1992; Loughlin and York, 2000). It is likely that changes in prey availability linked to commercial fisheries and large-scale oceanographic changes are among the reasons for the continued decline (Loughlin and Merrick, 1989; NRC, 1996). The diet of the western stock of Steller sea lions has been recently assessed (Sinclair and Zeppelin, 2002), but discrete selection of prey by size has not been described. The size of prey is relevant for understanding the foraging behavior of the predator as well as the ecological role of the prey (e.g., mortality at a given life history stage). In the case of the Steller sea lion, prey-size selectivity is particularly important for understanding spatial and temporal changes in diet and is needed for making fishery management decisions.

Size of fish prey consumed by marine mammals has been estimated by using sagittal otoliths recovered from stomach and more recently scat samples (Pitcher, 1981; Frost and Lowry, 1986; Browne et al., 2002). Significant relationships have been demonstrated between fish fork length (FL) and otolith length (Templeman and Squires, 1956; Frost and Lowry, 1981; Harvey et al., 2000). The use of otoliths to describe the size of prey taken by Steller sea lions has proved useful in data collected from stomach samples (e.g., Pitcher, 1981; Calkins and Goodwin[1]). However, few

[1] Calkins, D. G., and E. Goodwin. 1988. Unpubl. report. Investigation of the declining sea lion population in the Gulf of Alaska, 76 p. Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, Alaska, 99518-1599.

otoliths are recovered from Steller sea lion scat, and measurements of otoliths recovered from scats likely underestimate prey size because of partial erosion from digestion (Prime and Hammond, 1987; Dellinger and Trillmich, 1988; Harvey, 1989). Because of the impracticality of collecting stomachs and the low number and poor quality of otoliths found in scats, alternative methods are needed to accurately describe the size of prey consumed by Steller sea lions.

Archaeological studies routinely use skeletal structures other than otoliths to estimate either fish length or mass (Keys, 1928; Casteel, 1976; Owen and Merrick, 1994; Desse and Desse-Berset, 1996). Wise (1980) used a regression of fish length on vertebrae length to estimate prey size from scat samples of otters (*Lutra lutra*) and mink (*Mustela vison*). The regression approach relies on the assumption that the overall size of a given fish and the size of skeletal structures are highly correlated. This assumption has been substantiated for cranial and skeletal structures other than otoliths in various North Pacific fish species (Orchard, 2001). Thus, the use of cranial structures appear to be a viable alternative to the use of otoliths for studying prey size of Steller sea lions.

Walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius*) rank among the top prey items of Steller sea lions (Sinclair and Zeppelin, 2002) as well as being valuable in the U.S. commercial fishery (NMFS, 2003). We estimated fork length for these two primary prey species from scats collected between 1998 and 2000 across the range of the Alaskan western stock of sea lions. Fish length was estimated by using regression formulae relating bone or otolith measurement to fork length for seven cranial structures found in sufficient quantities and in good and fair condition in scat samples. Experimentally derived digestion correction factors (Tollit et al., 2004b, this issue) were applied to bone and otolith measurements to account for loss of size due to erosion. The methods developed here proved to be an effective tool to estimate size of prey selected by Steller sea lions and are applicable for other marine mammal diet studies particularly where otoliths are highly eroded.

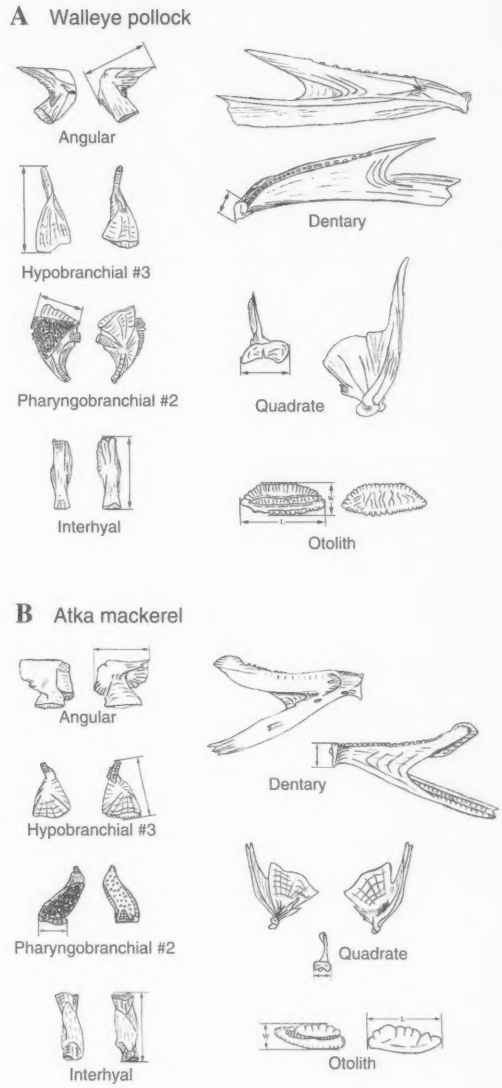

Figure 1

Illustrations of the various planes for bone and otolith measurements used to solve the bone-length to fish-length regression equations for (**A**) walleye pollock and (**B**) Atka mackerel. The structures from the right side of the body are shown for all structures except for quadrates.

Materials and methods

Development of regression formulae

Fork-length to bone and otolith-length regression equations were developed for seven cranial structures from walleye pollock and Atka mackerel. Bones and otoliths were selected according to species-specific features, predictability in condition, and prevalence in scats. Bones included the angular (ANG), quadrate (QUAD), interhyal (INTE), dentary (DENT), pharyngobranchial 2 (PHAR), and hypobranchial 3 (HYPO) (Fig. 1). Fork length regressions were developed for sagittal otolith length (OTOL), as well as for width (OTOW) measurements. All selected cranial structures were paired (having a left and right side) which allowed for enumeration of prey species. Only right-sided bones and otoliths were used to develop the regression equations.

In symmetrical fishes such as walleye pollock and Atka mackerel the left and right otoliths are mirror images of each other (Härkönen, 1986). We compared the left and right-sided measurements for all seven structures using a subsample of the structures used to develop the regression equations. There was no significant difference for either walleye pollock (paired t-test, $P<0.05$, $n=13$ for HYPO, 15 for QUAD, and 14 for all other structures) or Atka mackerel (paired t-test, $P<0.05$, $n=14$ for OTOS and 17 for all other structures).

Fish specimens used for regressions were collected from the Gulf of Alaska and Bering Sea. Standard length (SL) was converted to fork length for walleye pollock (when fork length was not available for a small number of otoliths included in the regressions) by using the following equation: $FL = 0.40+1.07(SL)$ (Wilson[2]). We chose to use FL over SL for the regressions because all fish were in good condition, thus allowing for accurate measurements. Additionally, FL is the standard used for commercial fishery and survey data by the National Marine Fisheries Service for direct comparisons. A partial analysis of these data was previously reported in Orchard (2001). We expanded the data set reported in Orchard (2001) to reflect the size range of bones found in Steller sea lion scats and included only fish specimens collected within our study area.

Linear regression models were fitted for most cranial structures by using the following equation:

$$Y = \alpha + \beta X,$$

where Y = the fork length of the fish;
X = the measurement of the cranial structure; and
α and β are constants that define the regression formula.

However, some cranial structures provided a better fit with the following quadratic regression equation:

$$Y = \alpha + \beta X + \beta X^2.$$

The strength of the relationship of the regression models was assessed by using a coefficient of determination (r^2).

Erosion is a potential source of bias when estimating prey body size from digested otoliths (Prime and Hammond, 1987; Dellinger and Trillmich, 1988; Harvey, 1989). We used condition-specific digestion correction factors (DCFs) developed by Tollit et al. (2004b, this issue) to correct for the high degree of variation in the erosion of cranial structures. DCFs were obtained from feeding experiments on captive juvenile Steller sea lions by using a subsample of fish collected for the regression analysis (Tollit et al., 2004b, this issue). Selected cranial structures from three size groups of pollock (28.5–45.0 cm FL) and one size group of Atka mackerel (30–36 cm FL) were used to develop the DCFs.

Estimation of size of walleye pollock and Atka mackerel consumed by Steller sea lions in the Bering Sea and Gulf of Alaska

Steller sea lion scats were collected from 1998 to 2000 along most of the U.S. range of the Alaskan western stock. Scats were collected from rookery (breeding) and haul-out (nonbreeding) sites in summer (June–September) and haul-out sites in winter (February–March). We assumed that scats collected on summer rookery sites primarily represent the diet of adult females because adult males present on rookeries usually fast during this time. Juveniles of both sexes come ashore on rookeries during summer and undoubtedly are represented in the data, but to a lesser degree than adult females. Scats from juvenile Steller sea lions are more likely to be sampled on haul-out sites during summer, where juveniles make up the greatest proportion of individuals. Scats collected on summer haul-out sites or any winter site presumably represent a greater cross-section of ages and sexes than collections from rookeries during summer.

Scats were rinsed through nested sieves of 4.8-, 1.4-, 0.7-, and 0.5-mm mesh. Bones and otoliths were identified to the lowest possible taxon by using reference collection specimens. All recovered otoliths and selected bones identified as either walleye pollock or Atka mackerel were given a condition grade based on the degree of erosion (Tollit et al., 2004b, this issue). In general, cranial structures considered in "good" condition had little or no erosion, "fair" were moderately eroded (generally up to about 20%), and "poor" were heavily digested (Tollit et al., 2004b, this issue). All structures that were given a condition grade of "good" or "fair" were identified as being from the left or right side and measured to the nearest 0.01 mm with digital calipers. Cranial structures graded as "poor" were not measured and excluded from further analyses because of high observed intraspecific variation (Tollit et al., 1997; Tollit et al., 2004b, this issue).

Fork-length estimates with and without DCFs applied were calculated for each cranial structure and for all structures combined. Otoliths were treated separately because most diet studies currently rely on otolith length to estimate fish fork length. Ninety-five percent confidence intervals around all mean size estimates were calculated by using parametric bootstrapping procedures (Manly, 1997) in which error associated with the regression equation and resampling error resulting from variability within correction factors, and variability in scats were taken into account. Full details of the bootstrapping procedure are presented in Tollit et al. (2004b, this issue).

The same fish may be represented by multiple cranial structures within a scat; therefore, in order to avoid pseudoreplication, we selected a minimum number of individuals (MNI; Ringrose, 1993) for each scat sample.

[2] Wilson, M. 2003. Personal commun. Alaska Fisheries Science Center, Natl. Mar. Fish. Serv., NOAA, Seattle, WA.

Table 1

Relationship between bone measurement and fish fork length (FL) in millimeters. For each equation the number of bones measured (n), coefficient of determination (r^2), standard error of the regression coefficient (SE and SE_x^2 for quadratic regression coefficients), range of fish lengths and mean of fork lengths are given. All measurements are given in millimeters.

Species	Structure code	Regression	r^2	n	SE, SE_x^2	Range of FL	Mean FL
Walleye pollock	INTE	$FL = 49.78x + 5.12$	0.98	49	1.12	83–477	201.61
	HYPO	$FL = 43.14x + 14.12$	0.99	49	0.78	83–477	231.58
	PHAR	$FL = 80.19x + 19.43$	0.95	51	2.58	83–477	204.37
	ANGU	$FL = 59.25x + 15.27$	0.96	44	1.82	83–477	208.75
	QUAD	$FL = 89.47x + 6.77$	0.99	59	1.32	83–477	203.92
	DENT	$FL = 108.46x - 1.52$	0.99	60	1.75	83–477	206.61
	OTOL	$FL = 0.50x^2 + 15.74x + 13.3$	0.99	504	0.68, 0.34	49–530	187.35
	OTOW	$FL = 2.32x^2 + 44.74x + 3.73$	0.99	508	1.54, 0.19	49–530	188.66
Atka mackerel	INTE	$FL = 57.38x + 95.57$	0.86	106	2.26	185–500	355.37
	HYPO	$FL = 38.58x\ 80.64$	0.95	105	0.85	185–500	355.62
	PHAR	$FL = 81.32x + 70.40$	0.91	107	2.48	185–500	354.90
	ANGU	$FL = 58.38x + 73.86$	0.91	105	1.85	185–500	355.34
	QUAD	$FL = -8.90x^2 + 129.38x + 9.16$	0.96	108	7.07, 0.96	185–500	354.69
	DENT	$FL = -7.10x^2 + 115.83x - 21.68$	0.94	108	7.08, 0.73	185–500	354.69
	OTOL	$FL = 62.54x + 24.24$	0.83	165	2.19	185–500	349.82
	OTOW	$FL = 188.19x - 77.71$	0.78	170	7.71	185–500	350.09

Minimum number of individuals for each species in each scat was estimated by counting species-specific sided elements and choosing the greatest number of left or right elements. If more than one structure had the same number, the structure with the highest r^2 value in its regression on fork length (Table 1) was selected as a representative length estimate for that fish. If an equal number of left and right bones were present, right bones were selected.

Temporal variation in size of walleye pollock and Atka mackerel consumed by Steller sea lions

Temporal differences were assessed by grouping fish into stage-class categories. Stage-class categories were defined for pollock as follows: juvenile or 1-year-old fish (≤20 cm FL), adolescent (20.1–34 cm FL), subadult (34.1–45 cm FL), and adult (>45.1 cm FL; Dorn et al., 2001; Smith, 1981; Walline, 1983). Walleye pollock subadults are likely 3–4 years old, of which ~50% have matured and recruited into the fishery, whereas adults are sexually mature fish, likely 5 years or older. Stage-class categories for Atka mackeral were defined as follows: juvenile up to 2-year-old fish (≤30 cm), adolescent or 3-year-old fish (30.1–35.2 cm), subadult or 4-year-old fish (35.3–45 cm), and adults (>45.1 cm; Lowe et al., 2001; McDermott and Lowe, 1997). Atka mackerel adolescents are ~50% sexually mature and adult-size fish are fully mature.

A chi-squared contingency test was used to test for differences in the proportion of fish stage-classes occurring in scats among rookeries and haul-out sites, years, and seasons by using corrected fork-length estimates from all cranial structures (S-PLUS 2000, Mathsoft, Inc., Cambridge, MA). To avoid pseudoreplication, we used presence or absence of cranial elements of a stage class in a scat particilarily because multiple elements from the same stage-class within a sample may not be independent (Hunt et al., 1996). By using presence-absence data we also avoided the problems associated with the variability in passage and recovery rates of different size structures (Tollit et al., 1997). Because sample sizes were small, juvenile and adolescent walleye pollock stage classes and recruiting adult and adult Atka mackerel stage classes were combined for seasonal comparisons among years. Fisher's exact test was used for comparisons when samples sizes for any stage class were less than 5 (S-PLUS 2000, Mathsoft, Inc., Cambridge, MA).

We obtained size composition data from commercial bottom trawls of walleye pollock and Atka mackerel from the NMFS North Pacific Groundfish Observer Program. Data were divided into winter (January–April) and fall (August–November) seasons and compared with our seasonal scat data (February–March and June–September). The percentage of overlap in sizes of fish caught by the commercial groundfish fishery with sizes of fish consumed by Steller sea lions was calculated by comparing size-frequency distributions. Two-cm size bins were used for the overlap calculation and Steller sea lion prey-size data were rounded to the nearest integer to be consistent with the fishery data.

Results

Regression formulae

A total of 517 pollock and 191 Atka mackerel samples were used to develop the regression equations of bone and otolith measurement to fork length. The sample size and range of fish lengths used for the regressions varied between species and cranial structures (Table 1). No clear indications of sample size required for regression analysis are currently provided in the literature; however, Owen and Merrick (1994) recommend a minimum sample size of 30–40. Sample sizes used to develop equations presented here ranged from 44 to 508.

In general, linear models were used for regression equations; however, several cranial structures were best fitted with a quadratic model. For both species, all structures were strongly related to fork length (r^2 range: 0.78–0.99; Table 1). The regressions encompassed the majority of sizes of bones and otoliths found in Steller sea lion scat samples for this study. However, a small proportion of walleye pollock bones from scats were larger than those used to develop the regressions.

Frost and Lowry (1981) developed otolith linear regression equations for walleye pollock from the Bering Sea using a double-regression approach that produced an inflection point at 10 mm. We examined the double regression approach but found a higher degree of correlation using a quadratic regression model. We compared the results of our model with Frost and Lowry's (1981) model and found that estimated fork lengths of walleye pollock differed less than 2 cm across the length range in our samples.

Estimation of size of walleye pollock and Atka mackerel consumed by Steller sea lions in the Bering Sea and Gulf of Alaska

A total of 714 scats from 39 sites contained 3646 selected cranial elements from either walleye pollock or Atka mackerel. Of those, 212 scats contained 666 walleye pollock cranial elements with a condition grade of either "good" (n=236) or "fair" (n=430). The minimum number of individual pollock per scat ranged from 1 to 18 with a mean of 1.6 (SD=1.7). For Atka mackerel, 379 scats contained 1685 skeletal elements with condition grade of either "good" (n=755) or "fair" (n=930). The minimum number of individual Atka mackerel per scat ranged from 1 to 14 with a mean of 1.9 (SD=1.6).

The mean fork length of walleye pollock consumed by Steller sea lions in the Bering Sea and Gulf of Alaska estimated from uncorrected otoliths found in scats was 23.7 cm (SD=12.0; n=88). Application of the DCF increased the mean estimate to 28.4 cm (SD=14.75; n=88). The size distribution estimated from corrected otoliths had three modes: a major mode around 32 cm and minor modes around 5 cm and 13 cm (Fig. 2A). Confidence intervals for all grade-corrected estimates can be found in Table 1.

The mean fork length of walleye pollock estimated from all seven structures was 39.8% greater than the mean estimated from otoliths alone. The uncorrected mean was 33.1 cm. Applying the DCF increased the mean length of walleye pollock by 18.7% to 39.3 cm (paired t test, t_{665}=37.9, P<0.001). Mean grade-corrected size estimates for cranial structures other than otoliths ranged from 34.5 cm (PHAR) to 47.2 cm (HYPO) and 95% confidence intervals ranged from 25.2 to 50.6 cm (Table 2). The condition-specific DCFs increased length estimates between 6.8% (HYPO) and 28.3% (DENT). The size distribution estimated from all grade-corrected structures had three modes: a major mode around 44 cm and minor modes around 5 cm and 15 cm (Fig. 2A).

The mean fork length of Atka mackerel consumed by Steller sea lions in the Bering Sea and Gulf of Alaska estimated from uncorrected otoliths was 30.3 cm (SD=4.0; n=117). Application of the DCF increased the mean estimate to 34.7 (SD=4.8; n=117).

The mean fork length of Atka mackerel estimated from all structures (30.7 cm; SD=5.9 cm, corrected 32.3 cm; SD=5.9 cm, n=1685, paired t test, t_{1684}=39.1, P<0.001) was similar to the mean estimated from otoliths (6.9% less without a DCF and 1.3% less with a DCF; Fig. 2B). Mean grade-corrected size estimates for structures other than otoliths ranged from 26.6 cm (QUAD) to 34.2 cm (INTE) and 95% confidence intervals ranged from 24.0 cm (DENT) to 35.0 cm (INTE; Table 2). Use of the condition-specific DCFs increased length estimates between 2.1% (INTE) and 24.0% (DENT). Fork length estimates for all structures did not include PHAR because too few were recovered in scats in the feeding studies of captive Steller sea lions to develop a correction factor.

When mean prey size was calculated by using MNI, the mean corrected and uncorrected size estimate of both walleye pollock and Atka mackerel differed by less than 0.2 cm from estimates derived by using all structures. There was little difference in the standard deviations or distributions when MNI estimates were used compared with all structures (Table 2). Unsurprisingly, the use of MNI estimates did substantially reduce the sample size (336/666 for walleye pollock and 722/1685 for Atka mackerel).

Spatial and temporal variation in size of pollock and Atka mackerel consumed by Steller sea lions

No statistical difference was found in the proportion of pollock stage classes among years on summer rookery sites (P=0.29, χ^2=4.9, df=3) or winter haul-out sites (P=0.10; Fisher's exact test). Scats were collected only on summer haul-out sites during 2000. Although sample sizes were limited, we found significant differences in the proportion of pollock stage classes between summer rookery and haul-out scats (P=0.02; Fisher's exact test) and between summer and winter haul-out sites (P=0.018; Fisher's exact test) for year 2000. A greater proportion of juvenile pollock were found on summer haul-outs (64% juveniles, n=11 scats) than on summer rookeries (9% juveniles, n=132 scats) or winter haul-out sites (3% juveniles, n=69 scats, Fig. 3). No statistical difference

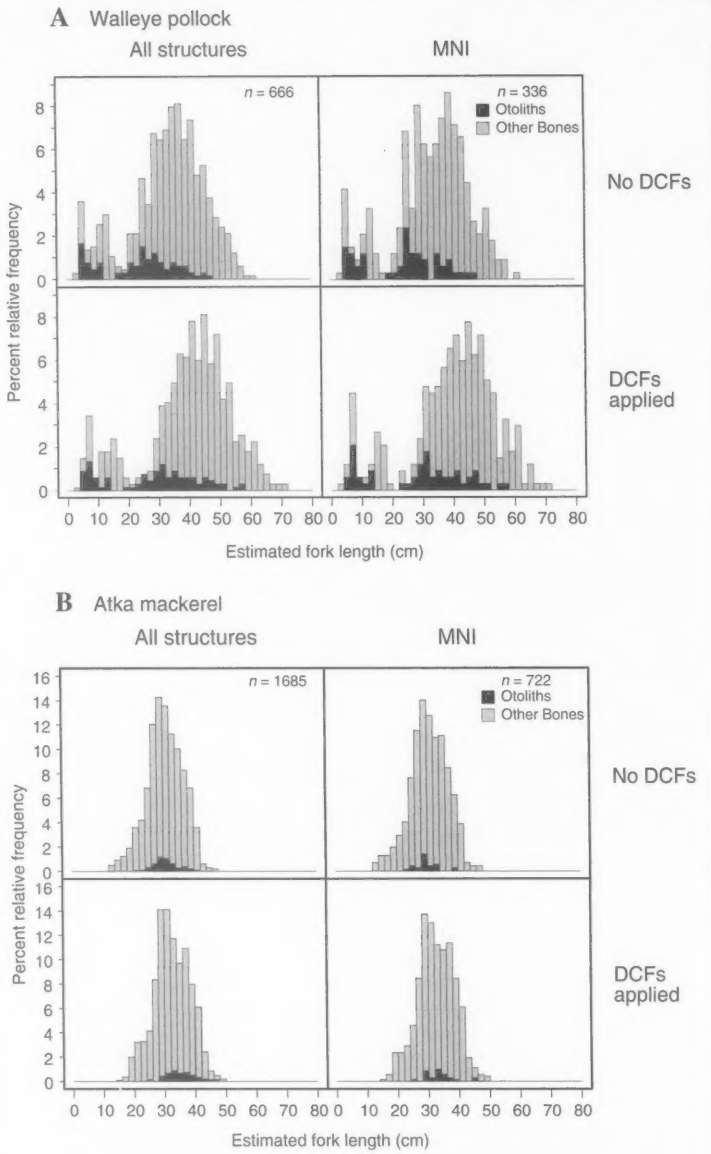

Figure 2

Relative frequency histograms of the estimated fork length of (**A**) walleye pollock and (**B**) Atka mackerel consumed by Steller sea lions. Fork lengths were predicted from cranial structures in good and fair condition. Comparisons were made on the application of correction factors (DCFs) which account for digestion and for using minimum number (MNI) estimates as a selection technique versus using all structures. Otoliths (black bars) are stacked beneath all other structures (gray bars).

was found in the proportion of stage classes between summer rookery (9.09% juvenile; 20.45% adolescent; 53.03% subadult; 65.15% adult) and winter haul-out (2.90% juvenile; 21.74% adolescent; 57.97% subadult; 46.38% adult) sites for all years combined (P=0.32, χ^2=2.3, df=2).

Table 2

Estimated mean fork length of walleye pollock and Atka mackerel consumed by Steller sea lions based on selected structures with or without application of condition-specific digestion correction factors (DCFs). Data sets exclude all structures graded as heavily digested. Remaining total sample sizes of elements (n^e) are given along with proportion of grade "good" structures (n^g). For data sets where DCFs were applied, 95% confidence intervals (95% CI) were estimated by using bootstrap resampling procedures (Tollit et al., 2004b, this issue).

Species	Structure code	DCF	n^e	n^g	Mean FL (cm)	SD (cm)	Range (cm)	95% CI
Walleye pollock	INTE	No	60	0.45	43.7	8.0	16.7–59.4	
		Yes	60	0.45	47.0	8.5	16.7–65.9	44.9–49.8
	HYPO	No	38	0.55	44.2	7.2	30.5–60.4	
		Yes	38	0.55	47.2	7.8	34.9–62.7	44.5–50.6
	PHAR	No	23	0.61	32.2	14.3	9.7–53.1	
		Yes	23	0.61	34.5	14.8	10.9–53.1	25.2–44.5
	ANGU	No	136	0.40	36.1	8.4	10.6–55.3	
		Yes	136	0.40	40.2	9.0	10.6–60.6	38.5–42.4
	QUAD	No	134	0.34	35.1	12.0	9.4–57.8	
		Yes	134	0.34	44.5	15.3	11.9–70.8	38.8–49.6
	DENT	No	187	0.37	28.6	11.8	3.1–57.2	
		Yes	187	0.37	36.7	15.1	3.7–70.2	30.3–42.4
	OTOL	No	88	0.03	23.7	12.0	4.6–46.8	
		Yes	88	0.03	28.4	14.8	4.6–57.1	17.0–32.4
	All	No	666	0.35	33.1	12.4	3.1–60.4	
		Yes	666	0.35	39.3	14.3	3.7–70.8	35.9–42.4
Atka mackerel	INTE	No	601	0.58	33.5	5.0	19.5–46.8	
		Yes	601	0.58	34.2	5.1	19.5–49.6	33.4–35.0
	HYPO	No	238	0.42	31.1	4.8	18.8–46.2	
		Yes	238	0.42	32.9	5.5	19.3–48.3	32.4–34.6
	ANGU	No	488	0.45	30.2	4.7	17.3–43.0	
		Yes	488	0.45	31.8	5.1	17.3–46.1	31.7–33.3
	QUAD	No	161	0.37	25.3	5.4	14.8–40.6	
		Yes	161	0.37	26.6	5.6	15.3–41.4	25.1–28.4
	DENT	No	80	0.28	22.5	7.7	13.0–38.7	
		Yes	80	0.28	27.9	8.0	17.7–44.1	24.0–33.0
	OTOL	No	117	0.06	30.3	4.0	21.2–40.6	
		Yes	117	0.06	34.7	4.8	21.2–47.0	33.5–35.8
	All	No	1685	0.45	30.7	5.9	13.0–46.9	
		Yes	1685	0.45	32.3	5.9	15.3–49.6	31.7–33.4

Significant differences were found in the proportion of Atka mackerel stage classes between 1998 and 1999 on summer rookery sites (P=0.05, χ^2 =6.0, df=2) and winter haul-out sites (P=0.01, χ^2=9.9, df=2) and between 1998 and 2000 winter haul-out sites (P=<0.01, Fisher's exact test). Significant seasonal differences were found only in 1998 (P=0.03, χ^2=7.1, df=2) which may be the result of the small sample size in winter 2000. In summer and winter, annual differences in size of Atka mackerel consumed by Steller sea lions corresponded to changes in the length distribution of Atka mackerel resulting from exceptionally strong year classes in 1995 and 1998 (Lowe et al., 2001). The 1995 year class is represented as a mode around 30 cm in 1998 (3-year-old fish), 35 cm in 1999 and >40 cm in 2000 (Fig. 4). The 1998 year class is represented most clearly as 2 year olds (mode 20–25 cm) in summer 2000 (Fig. 4). Strong annual modes found in our data match those recorded in surveys of Atka mackerel in the Bering Sea and Gulf of Alaska (Lowe et al., 2001).

For walleye pollock and Atka mackerel there was no difference in the mean size of fish caught by the commercial fishery among years (P>0.4, one-way ANOVA). There was a significant difference (P<0.05, one-way ANOVA) in the size of fish caught between seasons. This difference is likely due to aggregations of spawning adult fish caught during the roe fishery. In the winter there is a 56% overlap between the size of fish caught in the commercial pollock fishery and those taken by sea lions and a 54% overlap in the size taken by the Atka

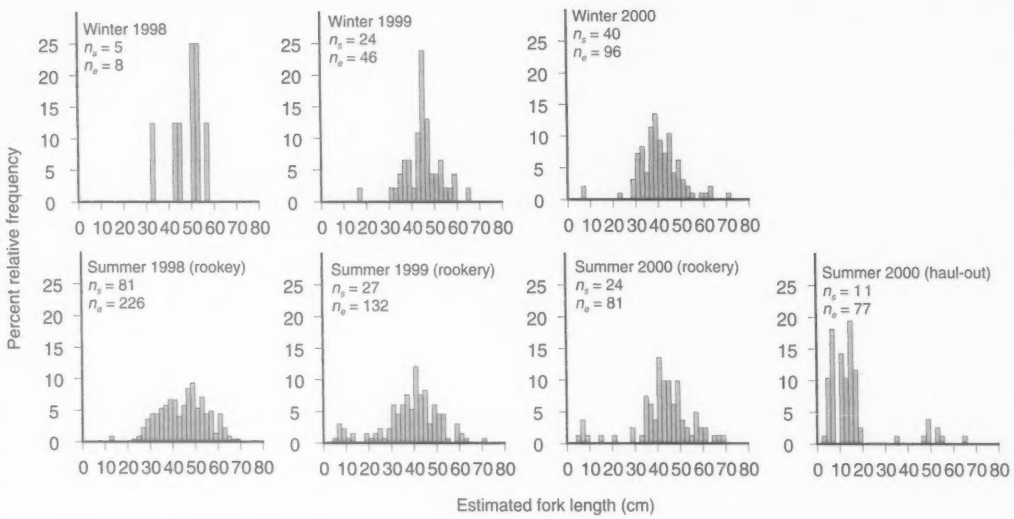

Figure 3

Relative frequency histograms of the estimated fork length of walleye pollock consumed by Steller sea lions across seasons and years for rookeries and haul-outs. Fork lengths are predicted from corrected cranial structures in good and fair condition. Sample sizes for cranial elements (n_e) and scats (n_s) are provided. All winter sites are considered haul-out sites.

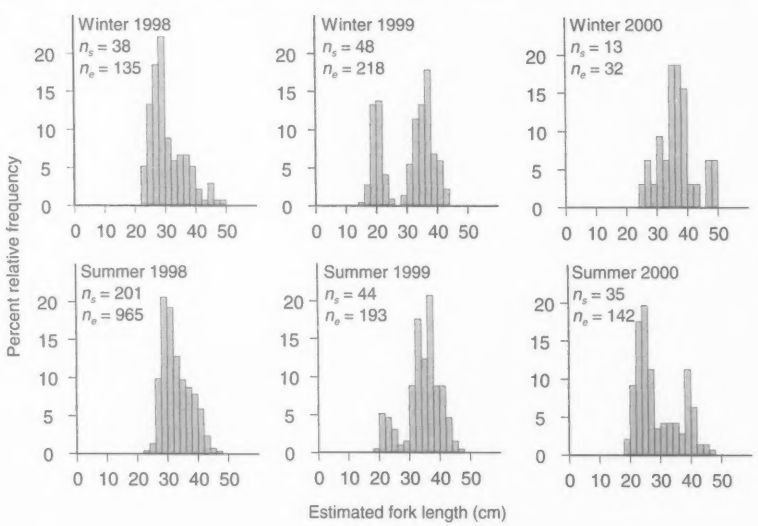

Figure 4

Relative frequency histograms of the estimated fork length of Atka mackerel of consumed by Steller sea lions by season and year. Fork lengths are predicted from corrected cranial structures in good and fair condition. Sample sizes for cranial elements (n_e) and scats (n_s) are provided. All summer sites are rookeries and winter sites are haul-out sites.

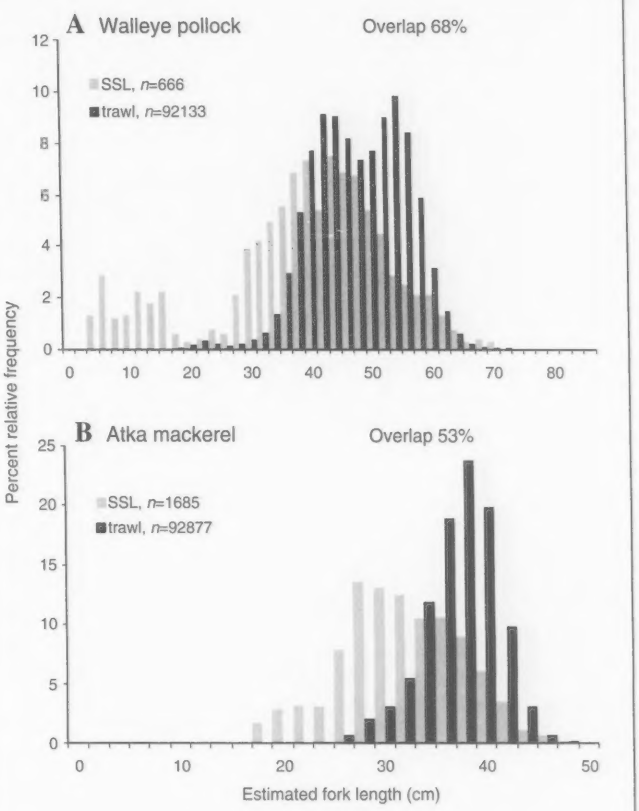

Figure 5

Relative frequency histograms of the estimated fork length of walleye pollock and Atka mackerel consumed by Steller sea lions (SSL) compared with relative frequency histograms of fish caught by the walleye pollock and Atka mackerel commercial trawl fishery.

mackerel fishery. In the summer the overlap in size of fish consumed by sea lions and the size of fish caught in the pollock fishery is 67% and there is a 51% overlap in the size of fish caught in the Atka mackerel fishery. When seasonal data were pooled, overlap between the size of fish caught in the commercial fishery and the size of fish consumed by sea lions was 68% for walleye pollock (Fig. 5A) and 53% for Atka mackerel (Fig. 5B).

Discussion

Regression formulae

Regressions of cranial structure measurement on fish fork length with the use of multiple structures was an effective tool for estimating size of fish consumed by Steller sea lions. Sample sizes of measurable prey remains from scats were enhanced by using a number of cranial structures in addition to otoliths. Body size estimates of only 13.2% of the pollock and 6.9% of the Atka mackerel prey in this study were based on otoliths alone. Fork-length estimates can be considered accurate regardless of which structure was used in the estimate because all r^2 values were high (range: 0.78–0.99). Likewise errors associated with the application of DCFs were consistent across structures (Tollit et al., 2004b, this issue). Confidence intervals around size estimates generally overlapped across structures; however, it was not surprising that different structures yielded slightly different mean sizes because different bones can originate from different scats.

The use of multiple cranial structures may also reduce bias resulting from variability in recovery and passage rates of structures from different species or sizes of fish (Pierce and Boyle, 1991; Browne et al.,

2002; Tollit et al., 2003). Even after applying a DCF, the estimated mean size of walleye pollock based on otoliths was 10.9 cm smaller than the mean size estimated by using all cranial structures. Because walleye pollock otoliths are relatively large and have a different composition than other cranial structures, the larger otoliths may be regurgitated, fully digested, or crushed by rocks in the stomach and not pass through in scat as readily as smaller otoliths or other cranial structures, thereby reducing their occurrence in scat and use in generating prey-size estimates. Atka mackerel otoliths are much smaller at older ages in relation to walleye pollock, which may explain why the size of prey estimated from otoliths was similar to the size estimated from other cranial structures.

The use of DCFs for all structures, including otoliths, to account for erosion increased mean size estimates for both walleye pollock (33.1 vs. 39.3 cm FL) and Atka mackerel (30.7 vs. 32.3 cm FL). The relatively small increase in the corrected size of Atka mackerel reflects that the structures from this species were found in better condition than those from pollock (Table 2), as well as that correction factors were found to be species-, structure-, and condition-specific (Tollit et al., 2004b, this issue). Overall, our results emphasize the importance of using appropriate condition-specific DCFs. Other studies with captive sea lions have also demonstrated that grade-specific DCFs can reduce systematic error and increase precision of body mass estimates (Tollit et al. 1997). For walleye pollock, there was no significant difference in the degree of erosion across the three size ranges for each structure within each condition category (Tollit et al., 2004b, this issue). We assume the DCFs can be used for fish outside of this size range because the relative shape, structure, and proportion of the morphological features are consistent for both smaller and larger fish (Tollit et al., 2004b, this issue). Further research is necessary to test whether there are differences across the size range for Atka mackerel.

Size of walleye pollock and Atka mackerel consumed by Steller sea lions in the Bering Sea and Gulf of Alaska

In general, Steller sea lions on summer rookery and winter haul-out sites consumed primarily subadult and adult-size walleye pollock and Atka mackerel year-round in 1998–2000. Steller sea lions typically forage near shore, in shallow water (<50 m) and at night (Raum-Suryan et al., 2002; Loughlin et al., 2003). Likewise, adult walleye pollock migrate vertically to shallower depths during the night (Smith, 1981). Adult-size Atka mackerel also are commonly found in nearshore coastal areas during their spawning season (Zolotov, 1993).

Juvenile walleye pollock were found in relatively high numbers only in scats collected on summer haul-out sites. Scats collected from summer haul-out sites likely represent a larger proportion of juvenile Steller sea lions than those collected on summer rookery or winter haul-out sites. Previous studies indicate that juvenile sea lions (<4 years old) consume smaller walleye pollock than adult sea lions (Pitcher, 1981; Frost and Lowry, 1986; Merrick and Calkins, 1996). Juvenile walleye pollock are often found at shallow depths in bays and near shore habitat (Smith, 1981). Likewise, Loughlin et al. (2003) reported that juvenile Steller sea lions are typically shallow divers and frequently make short range foraging trips (<15 km). Additional scat collections on summer haul-out sites are necessary to determine more conclusively prey-size selectivity for juvenile Steller sea lions.

Annual changes in the size-frequency distribution of Atka mackerel consumed by Steller sea lions followed changes in the size distribution of Atka mackerel resulting from exceptionally strong year classes. Merrick and Calkins (1996) also showed that the size of prey consumed by Steller sea lions can reflect the size distribution of the fish population. From the mid-1990s on, only 1999 was a strong recruitment year for walleye pollock in the Gulf of Alaska (Dorn et al., 2001), but we did not find a significantly greater proportion of juvenile fish eaten by Steller sea lions in 2000 than in 1999 or 1998 perhaps because sufficient numbers of larger size fish were available in regions where walleye pollock were consumed.

Historical studies of Steller sea lion prey size have primarily been based on measurements of walleye pollock otoliths found in stomach samples but often without application of correction factors for erosion (Pitcher, 1981; Merrick and Calkins, 1996; Calkins, 1998). Prey-size estimates based on stomach contents will likely differ from estimates derived from scats because of differences in digestion rates and breakage (Jobling and Breiby, 1986). However, results of studies examining the variability in prey size with sample type are variable. Sinclair et al. (1996) suggested that in northern fur seals (*Callorhinus ursinus*), another otariid, small otoliths tend to flush through the digestive system more quickly than larger ones, resulting in a possible bias in scats towards smaller otoliths. In contrast, experiments with captive sea lions have shown that smaller otoliths are recovered in lower relative frequencies than are larger ones (Tollit et al., 1997). Frost and Lowry (1980) found no significant difference between the size of otoliths obtained from stomach and intestines of ribbon seals. Overall, we believe useful comparisons of prey size consumed by Steller sea lions can be made between our study and earlier studies.

Steller sea lions have been reported to consume a wide size range of walleye pollock. However, in most prior studies a larger proportion of juvenile fish were found than what we estimated from scats. Otoliths from stomach samples collected from 1975 to 1978 in the Gulf of Alaska contained primarily juvenile age pollock (mean FL=29.8cm; SD= 11.6; Pitcher, 1981). Undigested otoliths from stomach samples collected between 1975 and 1981 in the Bering Sea also contained mostly juvenile fish (mean FL=29.3 cm) but had a distinct mode of adult-size pollock (48 cm FL; Frost and Lowry, 1986). Likewise, 43 stomach samples collected between 1985

and 1986 in the central Gulf of Alaska contained primarily juveniles (mean FL=25.4 cm; SD=12.4) and had a weak mode of adult-size fish (39–43 cm; Merrick and Calkins, 1996; Calkins and Goodwin[1]). Mostly adult-size fish were found in stomachs recovered from Steller sea lions caught in trawl nets in the central Gulf of Alaska (1983–84; Loughlin and Nelson, 1986) and in stomach samples collected from 1994 to 1995 in Japanese waters (Goto and Shimazaki, 1998). However, in both these studies the samples of prey size may have been biased by the selectivity of the fishing gear for larger fish.

Using identical methods to those of our study, Tollit et al. (2004a, this issue) estimated the size of walleye pollock consumed by the eastern stock of Steller sea lions between 1994 and 1999. The average size of walleye pollock consumed, estimated from all grade-corrected structures (mean=42.4 cm; SD=11.6), was similar to the average size found in our study of the western stock of Steller sea lions. Furthermore, Tollit et al. (2004a, this issue) also found a greater occurrence of adult pollock in scats collected on rookery sites than from scats collected on haul-out sites. However, Steller sea lions from the western stock consumed a greater proportion of juvenile and adolescent fish and less adult fish than those from the eastern stock during summer (June–July) and similar-size fish were consumed on haul-out sites in winter (March) in both regions. Neither study indicated the high occurrence of juvenile walleye pollock reported in the 1970s and 1980s. The greater occurrence of juvenile walleye pollock in historical studies may be a result of prey availability or differences in sampling methods.

By examining the relative size-frequency distributions of prey selected by Steller sea lions and those taken in the commercial trawl fishery, we found considerable overlap (68% walleye pollock and 53% Atka mackerel). Likewise, high levels of potential overlap in size were found between walleye pollock selected by Steller sea lions from the eastern stock and caught by the small commercial fishery bordering Southeast Alaska (Tollit et al., 2004a, this issue). The extent of overlap throughout the range of Steller sea lions between the size of prey consumed by sea lions and the size of fish targeted and taken by the pollock and Atka mackerel trawl fisheries could result in competition between fisheries and foraging sea lions if resources are limited.

Acknowledgments

Fish specimens for the regression equations were provided by the National Marine Fisheries Service, the University of Victoria, and the University of British Columbia bone reference collections. Fish remains were identified by Pacific Identifications, Victoria, BC. We thank J. Laake, A. York, and R. Joy for statistical advice, K. Chumbley, E. Sinclair, and S. Crockford for help in initiating the study, A. Browne and M. Wilson for walleye pollock otolith data, and S. Heaslip for graphics and laboratory assistance. Reviews by E. Sinclair, S. Melin, W. Walker, B. Robson, and three anonymous reviewers greatly improved this manuscript.

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Sizes of walleye pollock (*Theragra chalcogramma*) consumed by the eastern stock of Steller sea lions (*Eumetopias jubatus*) in Southeast Alaska from 1994 to 1999

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Abstract—Lengths of walleye pollock (*Theragra chalcogramma*) consumed by Steller sea lions (*Eumetopias jubatus*) were estimated by using allometric regressions applied to seven diagnostic cranial structures recovered from 531 scats collected in Southeast Alaska between 1994 and 1999. Only elements in good and fair condition were selected. Selected structural measurements were corrected for loss of size due to erosion by using experimentally derived condition-specific digestion correction factors. Correcting for digestion increased the estimated length of fish consumed by 23%, and the average mass of fish consumed by 88%. Mean corrected fork length (FL) of pollock consumed was 42.4 ±11.6 cm (range=10.0–78.1 cm, n=909). Adult pollock (FL>45.0 cm) occurred more frequently in scats collected from rookeries along the open ocean coastline of Southeast Alaska during June and July (74% adults, mean FL=48.4 cm) than they did in scats from haulouts located in inside waters between October and May (51% adults, mean FL=38.4 cm). Overall, the contribution of juvenile pollock (≤20 cm) to the sea lion diet was insignificant; whereas adults contributed 44% to the diet by number and 74% by mass. On average, larger pollock were eaten in summer at rookeries throughout Southeast Alaska than at rookeries in the Gulf of Alaska and the Bering Sea. Overall it appears that Steller sea lions are capable of consuming a wide size range of pollock, and the bulk of fish fall between 20 and 60 cm. The use of cranial hard parts other than otoliths and the application of digestion correction factors are fundamental to correctly estimating the sizes of prey consumed by sea lions and determining the extent that these sizes overlap with the sizes of pollock caught by commercial fisheries.

Manuscript submitted 28 April 2003 to Scientific Editor's Office.

Manuscript approved for publication 25 March 2004 by the Scientific Editor.

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The dramatic decline of the western population of Steller sea lions (*Eumetopias jubatus*) in the 1980s (Loughlin et al., 1992; Trites and Larkin, 1996) prompted a number of studies to determine what they eat and the extent of overlap of the fish consumed by Steller sea lions and fish caught by commercial fisheries. The eastern population of sea lions (east of longitude 144°) located mainly in Southeast Alaska and British Columbia gradually increased as the western population declined (e.g., Calkins et al., 1999), permitting insightful comparative studies to be undertaken (e.g., Merrick et al., 1995; Milette and Trites, 2003). Possible explanations for the different population trends include ocean climate, competition with fisheries, predation, and the amount or the sizes of pollock in the diets of sea lions in the two regions (Loughlin and York, 2000; Benson and Trites, 2002; NRC, 2003; Trites and Donnelly, 2003; Calkins and Goodwin[1]; Loughlin and Merrick[2]).

Reliable estimates of prey size are important not only to investigate prey selectivity and the extent of overlap in size of prey with size of the same species caught by commercial fisheries and by other marine piscivores but are also vital for accurately assessing prey numbers, biomass, and total consumption (Beverton, 1985; Ringrose, 1993; Laake et al., 2002). One means of estimating prey size is to measure hard parts recovered from fecal remains and to apply allometric regressions relating fork length to the size of otoliths (Frost and Lowry, 1981) and other bones (Zeppelin et al., 2004, this issue). However, the extent of digestion incurred by both otoliths and bones as they pass through the digestive tract must be accounted for to ensure that prey size is not underestimated (Tollit et al., 2004, this issue). Application of these two steps is integral to correctly estimate the size of prey consumed by Steller sea lions and other pinnipeds.

The goal of our study was to estimate the size of walleye pollock (*Theragra chalcogramma*) consumed by Steller sea lions in Southeast Alaska between 1994 and 1999 by using new methods outlined by Tollit et al. (2004, this issue) and Zeppelin et al. (2004, this issue). Previous size estimates for this region of Alaska are based on the analysis of only eight stomachs collected in 1986 (Calkins

[1] Calkins, D. G., and E. Goodwin. 1988. Unpubl. report. Investigation of the declining sea lion population in the Gulf of Alaska, 76 p. Alaska Department of Fish and Game, 333 Raspberry Rd, Anchorage, AK 99518.

[2] Loughlin, T. R., and R.L. Merrick. 1989. Comparison of commercial harvest of walleye pollock and northern sea lion abundance in the Bering Sea and Gulf of Alaska. *In* Proceedings of the international symposium on the biology and management of walleye pollock, Nov. 14–16, 1988, Anchorage, AK, p. 679–700. Alaska Sea Grant Rep. 89-01, Univ. Alaska Fairbanks, Fairbanks, AK.

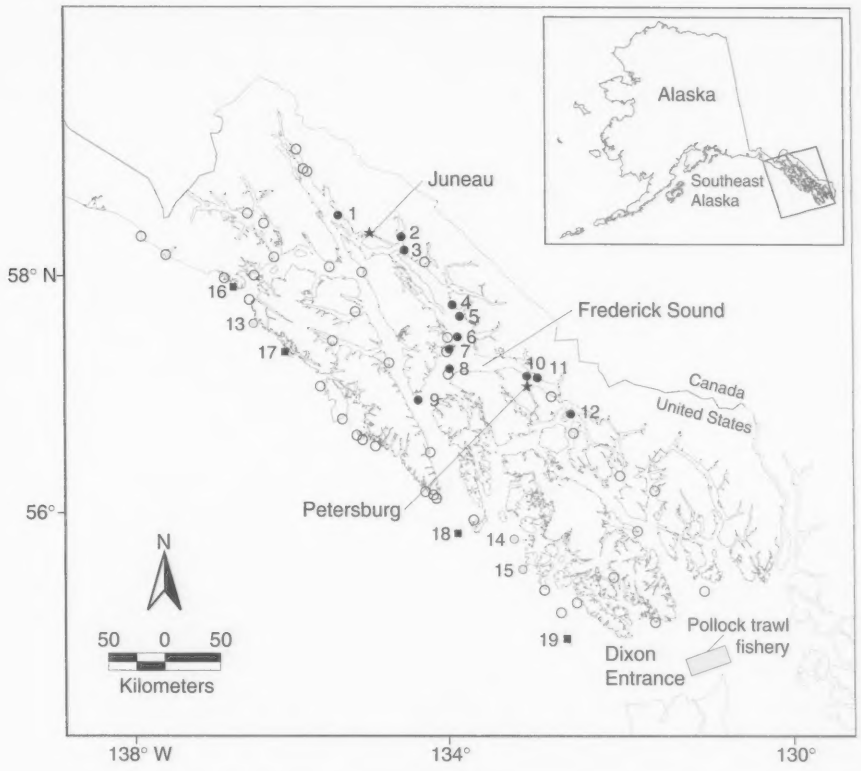

Figure 1

Location of Steller sea lion (*Eumetopias jubatus*) haul-outs and rookeries visited during 1994–99 to collect scats containing pollock hard remains. Symbols: haul-outs in inside waters (●), haul-outs in outside waters (●), haul-outs where scats were not collected or sites at which no pollock hard remains were found (○), rookeries (■), and cities (★).

and Goodwin[1]). We sought to compare the sizes of pollock consumed in the 1990s with these earlier samples, as well as with the sizes consumed by the declining population of sea lions in the Gulf of Alaska and Bering Sea during the 1970s and 1980s (e.g., Pitcher, 1981; Merrick and Calkins, 1996) and between 1998 and 2000 (Zeppelin et al., 2004, this issue). We also wanted to evaluate the use of digestion correction factors (DCFs) and skeletal structures other than otoliths to estimate prey size, and to compare the different size estimates for fish consumed by sea lions in Southeast Alaska with sizes of fish caught by a nearby commercial trawl fishery.

Materials and methods

Estimating sizes of pollock consumed

Scats that contained pollock hard remains were collected from four rookeries and 16 haul-outs from both inside and outside waters of Southeast Alaska between 1994 and 1999 (Fig. 1 and Table 1). Scats from three haul-outs and four rookeries in outside waters were collected from May through October 1994–99, but most were collected from June and July. Scats from inside waters were collected at 13 haul-outs located in the straits and sounds between Juneau and Petersburg, Alaska (56.8–58.6°N, 132.8–134.9°W) (Fig. 1). The majority of these "inside" scats were collected from Frederick Sound (Fig. 1) between October 1995 and February 1997. Most were collected in the winter and spring, but some were collected in the summer of 1999 (Trites et al.[3]). In general, the haul-out sites visited to collect scats were those with relatively high numbers of animals across Southeast Alaska (Calkins et al., 1999; Sease et al., 2001).

Scats were washed and sieved (0.5 mm) and hard remains were identified by Pacific IDentifications Inc. (Univ. of Victoria, Victoria, B.C.). Seven commonly

[3] Trites, A. W., D. G. Calkins, and A. J. Winship. 2003. Unpubl. data. Marine Mammal Research Unit, Fisheries Centre, University of British Columbia, Room 18, Hut B-3, 6248 Biological Sciences Rd., Vancouver, B.C., Canada, V6T 1Z4.

Table 1

Steller sea lion scat collection sites in Southeast Alaska, as illustrated in Figure 1, giving details of the type (HO=haul-out), fish element sample size (n_f), and the estimated corrected mean fork length (mean FL, cm) of walleye pollock, based on seven cranial structures found in scats at each site.

Region	Site no.	Site name	Type	n_f	Mean FL	SD
Inside waters	1	Benjamin Island	HO	11	39.7	13.9
	2	Dorothy Island	HO	3	38.6	13.6
	3	Circle Point	HO	31	45.1	13.7
	4	Point League	HO	37	42.9	9.9
	5	Sunset Point	HO	196	37.4	10.3
	6	Sail Island	HO	36	35.5	10.0
	7a	W Brother Island	HO	8	40.9	14.7
	7b	SW Brother Island	HO	152	37.1	9.9
	8	Turnabout Island	HO	34	47.8	11.0
	9	Yasha Island	HO	19	44.8	5.3
	10	Sukoi Islets	HO	14	26.9	7.8
	11	Horn Cliffs	HO	19	44.3	12.7
	12	Liesnoi Island	HO	7	31.8	10.2
Outside waters	13	Cape Cross	HO	7	45.3	3.0
	14	Timbered Island	HO	5	39.3	8.7
	15	Point Addington	HO	1	53.5	—
	16	Graves Rock	Rookery	49	42.9	7.7
	17	White Sisters	Rookery	33	43.4	9.6
	18	Hazy Islands	Rookery	54	45.4	8.7
	19	Forrester Islands	Rookery	193	51.4	10.0

occurring, robust, and diagnostic pollock structures were removed from all scats containing pollock (see Tollit et al., 2004, this issue). All were from the cranium region (see Zeppelin et al., 2004, this issue) and included the sagittal otolith (OTO), as well as the interhyal (INTE), hypobranchial 3 (HYPO), pharyngobranchial 2 (PHAR), angular (ANGU), quadrate (QUAD), and the dentary (DENT). Each individual fish element was assigned one of three condition categories (good, fair, or poor) and was measured three times (±0.01 mm) at a specific location to calculate a mean estimate (see Tollit et al., 2004, this issue).

Fork lengths of pollock eaten by Steller sea lions in Southeast Alaska were first estimated by applying allometric regressions (Zeppelin et al., 2004, this issue) to otolith lengths (OTOL) without correcting for partial digestion (see Pitcher, 1981; Merrick and Calkins, 1996). We also measured and substituted otolith width (OTOW) when otoliths were broken lengthwise. We then applied appropriate DCFs and regression formulae to otoliths assigned in good and fair condition (Tollit et al., 2004, this issue). Finally, we applied allometric regressions (Zeppelin et al., 2004, this issue) to all elements of the remaining six cranial structures (bones) assigned to good or fair condition categories to provide estimates of fish size across structures both with and without applying the appropriate DCFs (Tollit et al., 2004, this issue). Structures in poor condition were excluded because of large intraspecific size variation noted from feeding experiments with captive sea lions (see also Sinclair et al., 1994; Tollit et al., 1997; Tollit et al., 2004, this issue).

To incorporate the major sources of error in our method, we calculated confidence intervals (95%) for fork-length estimates. First, we applied a random bootstrapped regression equation, followed by a bootstrapped correction factor applicable to each selected structure (see Tollit et al., 2004, this issue). For the five structures (INTE, HYPO, PHAR, ANGU, and OTO) in good condition for which Tollit et al. (this issue) recommended a DCF of 1.0 (no correction), we drew bootstrapped values from a discrete declining triangular probability distribution (◺) ranging from 1.0 to 1.05 (to simulate a limited degree of digestion). Finally, we bootstrapped individual scats at random, by selecting n scats with replacement from the original sample size n (to account for resampling variability across scats) and included only selected elements within those randomly bootstrapped scats. Bootstrap randomizations for these steps were done 1000 times and 95% confidence intervals were taken as the 25th and 975th values of the sorted bootstrapped values.

Finally, consideration was also given to whether an individual fish might be represented by different structures within a single scat. We therefore compared length estimates using all structures with those esti-

mated with the minimum number of individuals (MNI) technique (Ringrose, 1993; Browne et al., 2002). This technique is used to select structures within each scat that preclude pseudoreplication or double counting of fish. Within each scat, the structure with the greatest MNI was selected, and right-sided structures were selected over left-sided structures if both sides were found in equal number because right-sided structures are used in regression formulae. If two structures had the same MNI estimate, then selection was made on the structure with the larger regression determination coefficient, r^2 (OTO-W>OTO-L>QUAD>DENT>HYPO>INTER>ANGU>PHAR).

Geographical and temporal variation in sizes of prey consumed

All elements from the seven cranial structures in good or fair condition were used to compare size of pollock consumed by Steller sea lions in Southeast Alaska between regions (inside haul-outs versus outside rookeries), across years and across rookeries (with rookery data collected in June and July), and across months (with data collected from inside haul-outs). Biologically meaningful differences in FL of pollock were assessed by grouping corrected lengths into stage-class categories (juvenile or 1-year-old fish FL≤20 cm; adolescent 20<FL≤34 cm; subadult 34<FL≤45 cm; and adult FL>45 cm) (Smith, 1981; Walline, 1983; Dorn et al., 2001). Adults were considered to be mature fish ≥5 years old and targeted by fisheries (Smith, 1981). Subadults were likely 3 or 4 years old, of which only a proportion had matured or were targeted by the fishery. To avoid the possibility of pseudoreplication in our chi-squared comparisons, we used only the presence or absence of structures of each stage class in a scat because individual fish eaten by a sea lion may have come from an age-specific school and were therefore not independent (Hunt et al., 1996). Presence-absence data was chosen over MNI data because the former greatly reduces potential concerns regarding size-dependent recovery of cranial structures (Tollit et al., 1997). With the exception of our regional comparison, data from juvenile and adolescent stage-classes were pooled because of the low sample sizes of juvenile fish. A Fisher's exact test was used as an alternative test to chi-square comparisons when counts for a stage-class grouping were <5 (S-PLUS 2000, Mathsoft Inc., Seattle, WA).

Overlap of prey size with size of fish caught by fisheries

To assess the impact of using the new methods described and to compare the size of pollock consumed by sea lions with the size of pollock typically caught by fisheries, we obtained randomly subsampled size-frequency landing data from the Canadian commercial pollock fishery in Dixon Entrance (1993–1999) (Saunders[4]). This area is 115–135 km SE of the Forrester Island rookery on the southern border of Southeast Alaska (Fig. 1).

Results

Sizes of pollock consumed

The traditional method of estimating prey size from otoliths alone was not satisfactory because most otoliths were in poor condition (86%, n=247) or were broken lengthwise (>89%) (or were both broken and in poor condition). Cranial bones, on the other hand, occurred in higher numbers than otoliths and were therefore more useful for estimating prey size (Table 2).

Sixty-one percent of scats (1215 of 1987) collected from Southeast Alaska (1994–99) contained pollock remains, with an average MNI of 1.57 ±1.66 individual pollock per scat (range: 1–37 individuals). Many scats contained hard parts that were not useful for estimating prey size (e.g., gill rakers), leaving 531 scats (26%) with measurable selected structures. Of these, 303 scats contained 1746 elements in good (n=225), fair (n=684), and poor condition (n=837).

Applying digestion correction factors had a considerable effect on the estimated length and mass of fish consumed, and on the proportion that were deemed to be adults (Fig. 2). The estimated lengths of pollock calculated from all structures graded in good or fair condition (without accounting for digestion) was 34.4 ±9.7 cm (n=909, modal range: 32–40) (Table 2, Fig. 2). Lengths increased by 23% on average when appropriate DCFs were applied to each structure to account for the observed degree of digestion (mean FL=42.4 ±11.6 cm, modal range: 44–52, 95% CI=41.0–43.9) (paired t-test, t_{908}=67.1, P<0.001). A DCF of 1.0 (no correction required to account for digestion) was applied to 62 elements in good condition, resulting in a mean fork length of 39.6 ±11.9 cm estimated from those bones.

The size-frequency distribution of pollock consumed by sea lions also varied significantly following the application of DCFs (Kolmogorov-Smirnov, KS=176.2, P<0.001) and led to an increase in the proportion of fish thought to have been adult (>45 cm FL) from 16% to 44%. This result in turn reduced the proportion of fish thought to have been subadults (29%), adolescents (25%), and juveniles (<2%, ≤20 cm FL) (Fig. 2). The size range of pollock eaten ranged widely regardless of whether DCFs were applied (10–78 cm) or not (10–64 cm). When we calculated fork lengths using only elements selected according to MNI criteria, the means increased by just 0.5 cm for corrected and by just 0.3 cm for uncorrected lengths, with near identical standard deviations and distributions (Fig. 2) (Kolmogorov-Smirnov, uncorrected KS=0.33, P=0.89, corrected KS=0.032, P=0.91).

The use of all otoliths regardless of digestion state resulted in a mean fork length that was only about half of that derived by using all structures corrected for digestion (Table 2). Excluding otoliths in poor condition significantly reduced sample size (Table 2) but

[4] Saunders, M. 2002. Unpubl. data. Fisheries and Oceans Canada, 3190 Hammond Bay Road, Nanaimo, B.C., Canada, V9T 6N7.

Table 2

Estimated mean fork length (mean FL, cm) of walleye pollock consumed by Steller sea lions. Values were determined by using selected cranial structures with or without the application of condition-specific digestion correction factors (DCFs). Data sets exclude all structures graded in poor condition (with the exception of data sets marked with an asterisk). Fish element sample sizes (n_f) are given along with proportion of elements assigned condition category good (n^g). When DCFs were applied, 95% confidence intervals (95% CI) were estimated by using bootstrap resampling (see "Materials and methods").

Structure code	DCF	n	n^g	Mean FL	SD	Range	95% CI
INTE	No	37	0.35	44.0	8.0	28.0–54.5	—
	Yes	37	0.35	48.0	9.3	31.9–62.2	45.0–52.2
HYPO	No	47	0.19	35.3	8.9	19.0–52.0	—
	Yes	47	0.19	39.8	10.1	19.0–60.4	36.7–43.6
PHAR	No	20	0.25	38.1	8.5	20.4–50.3	—
	Yes	20	0.25	43.7	9.5	20.4–56.1	39.9–48.4
ANGU	No	207	0.16	34.0	10.2	10.0–62.8	—
	Yes	207	0.16	39.4	11.4	10.0–63.2	37.4–41.5
QUAD	No	238	0.36	33.1	10.4	14.0–63.8	—
	Yes	238	0.36	41.9	13.2	17.3–78.1	39.5–44.7
DENT	No	326	0.24	34.9	8.1	11.0–63.0	—
	Yes	326	0.24	45.1	9.8	14.7–75.3	43.3–46.8
OTOL	No	10	0.10	30.6	13.8	14.2–54.8	—
	Yes	10	0.10	36.6	17.6	16.7–67.2	27.0–51.1
OTOL or OTOW	No	34	0.03	27.2	16.1	10.8–54.8	—
	Yes	34	0.03	33.7	12.8	13.3–67.2	29.5–39.5
All structures	No	909	0.25	34.4	9.7	9.8–63.8	—
	Yes	909	0.25	42.4	11.6	10.0–78.1	41.0–43.9
OTOL*	No	27	0.04	23.3	11.9	7.9–54.8	—
OTOL or OTOW*	No	247	<0.01	20.2	9.7	5.0–58.0	—

increased our estimate of fork length by approximately 33%. Applying grade-specific DCFs increased these estimates by another 19% (to 36.6 cm) for otolith length and by 24% (to 33.7 cm) for a combination of otolith length and width (Table 2). All six remaining structures in good or fair condition provided larger corrected mean length estimates than did otoliths alone, but 95% confidence intervals derived from otoliths did overlap with other structures (Table 2). The smaller estimate provided by otoliths may reflect that >83% of grade corrected otoliths (n=34) came from the inside haulout sites, where animals seem to eat smaller fish (see following section). On the other hand, large pollock otoliths were observed to have been regurgitated in feeding studies on captive sea lions and also may be more easily crushed by rocks often found in the stomachs of Steller sea lions (Tollit et al., 2003). Regression formulae used in our study to predict pollock FL from otolith length were similar to those of Frost and Lowry (1981) for juvenile fish (<10 mm otolith length) but led to smaller fish size estimates (~1–1.7 cm) over the range of 30–50 cm.

The lengths of the biggest fish (corrected mean FL=48.0 cm) were estimated from measurements of INTE (Table 2), the structure with the lowest DCF. Dentary bones (the most abundant structure recovered) predicted mean (Table 2) and modes (FL 44–50 cm) similar to those predicted from all structures. Applying DCFs increased our length estimates by between 9% (INTE) and 29% (DENT) (paired t-tests, all P<0.001). Overall, corrected fork length estimates from elements in good condition were similar to those from elements in fair condition (Mann-Whitney U, P=0.47), but multiple comparisons indicated a significant difference (P<0.05) between condition categories for INTE and DENT (lengths were estimated to be longer from elements graded in fair condition).

Repeat measurements of individual elements were all within 0.04 mm of the mean, and 88.9–99.5% were within 0.02 mm. The highest variability was associated with ANGU, HYPO, and PHAR with 88.9%, 91.7%, and 94.9% of their respective measurements falling within 0.02 mm. A 0.02-mm measurement difference corresponded to only a 0.1–0.2 cm difference in fish length, depending on the structure used.

Small differences in estimates of fork lengths can have large effects on estimated body mass (given the exponential mass-length relationship, see Tollit et al., 2004, this issue) and can increase the mean mass of fish by more than sixfold depending on which method is used to estimate body length (all otoliths and no correction versus condition-corrected structures). The ap-

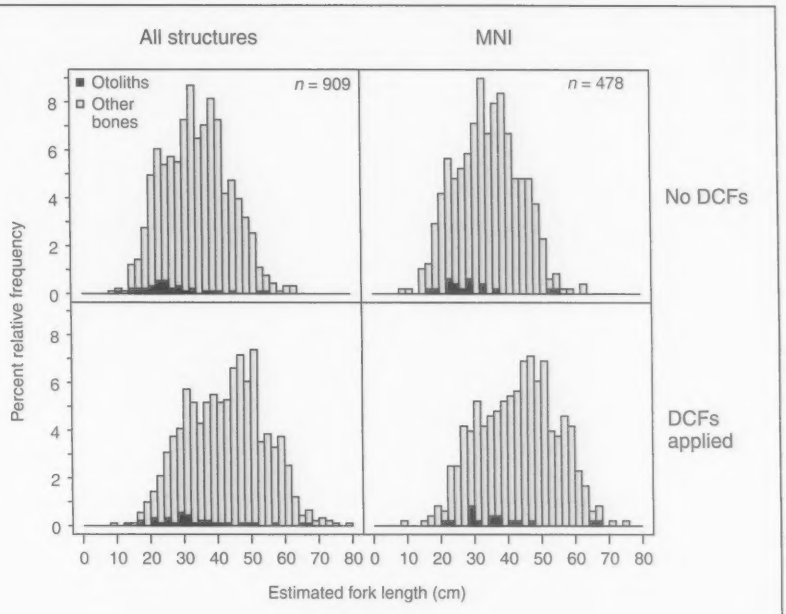

Figure 2

Relative frequency histograms of the estimated fork length of walleye pollock (*Theragra chalcogramma*) predicted from seven cranial structures in good and fair condition. Otoliths (black bars) are stacked beneath all other selected structures (gray bars). Comparisons were made with the application of digestion correction factors (DCFs) to take account of partial digestion and with the use of a selection technique (MNI) versus using all structures present in scats.

plication of our DCFs to all structures in good and fair condition increased the estimated mean mass of pollock consumed by 88% (from a mean of 388 g to 731 g). Thus, although we estimate that 44% of the number of pollock eaten by Steller sea lions were adults, based on length, their contribution, based on weight, increased to 74%. In contrast, the contribution of juvenile fish (in terms of mass) dropped to <0.1% (compared to <2% by number).

Geographical and temporal variation in sizes of pollock consumed

More pollock elements in good and fair condition were recovered from inside haul-outs (n=567) than from sites on the outside coastline (n=342) (Table 1). Upon investigation, we found the size of pollock consumed by Steller sea lions varied over time and across regions (Fig. 3). In particular, the frequency of occurrence of pollock stage classes differed significantly in the scats of sea lions resting at rookeries on the outside coastline of Southeast Alaska in summer (mean FL=48.4 cm, n=328, modal range: 44–50 cm, 95% CI=46.5–50.2, n_s=126 scats) compared to those collected between October and May at haul-outs in the waters of the inside passages (mean FL=38.4 cm, n=499, modal range; 30–34 cm, 95% CI=36.9–40.3, n_s=168 scats) (χ^2=45.2, P<0.001). Scats from these inside haul-outs contained a greater diversity of stage classes, and there was an equal probability of any given scat containing adults (51.2%), subadults (47.6%), and adolescents (53.0%), but a far less probability of containing juveniles (6.5%). In contrast, the pollock found in scats from the outside rookeries contained mostly adults (73.8%) and fewer occurrences of the remaining three stage classes (38.1%, 9.5%, and 3.2%, respectively). Notably, the stage-class comparison of summer 1999 with scats from inside versus outside waters was not significant (Fishers exact test, P=0.11).

Similar proportions of each pollock stage class were found in scats collected between years (Forrester, Fisher's exact test, P=0.54; Hazy, Fisher's exact test, P=0.16), and between rookeries (1994 only, Fisher's exact test, P=0.57; all years, Fisher's exact test, P=0.22). Scats from inside haul-outs collected in spring 1996 contained comparatively smaller fish than in other months and years examined (Fig. 3). However, there were no significant monthly differences in the proportions of age classes from October 1995 to February 1997 (χ^2=16.52, P=0.28) or when all monthly data (June and July 1999) were included from inside haul-outs (χ^2=23.4, P=0.10).

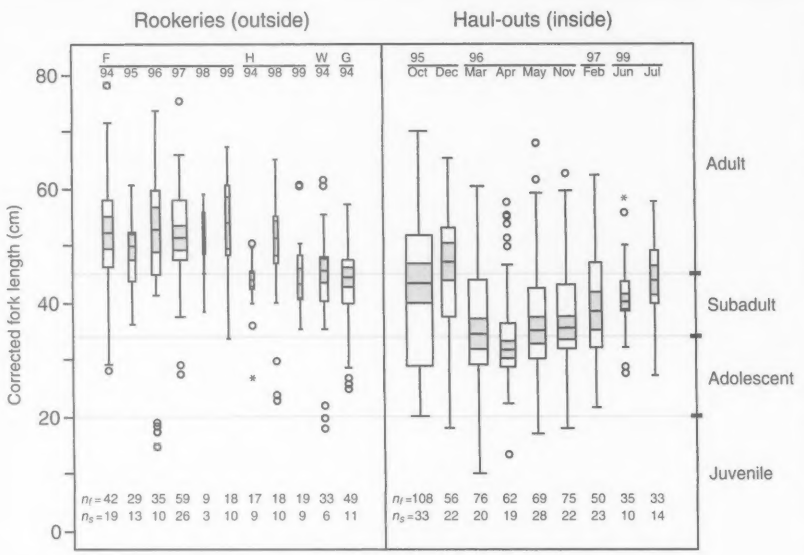

Figure 3

Box plots of corrected fork length of walleye pollock (*Theragra chalcogramma*) across months and years for haul-outs and rookeries in Southeast Alaska (Forrester Island, F; Hazy, H; White Sisters, W; Graves Rock, G). Box widths correspond to relative sample sizes for numbers of fish elements (n_f) and numbers of scats (n_s) are also provided, and stage-class categories are illustrated. Gray areas represent 95% confidence intervals, the whiskers bound 1.5× the interquartile range (boxes), the circles denote outliers, and the stars (*) denote extremes.

Overlap of size of pollock consumed by Steller sea lions with size of pollock caught by the fisheries

The Canadian commercial pollock trawl fishery in Dixon Entrance between 1993 and 1999 landed mostly (93%) adult fish (mean FL=52.2 ±5.9 cm, n=2103, modal range: 48–54 cm). The majority (79%) of scats containing pollock from the Forrester Island rookery in June and July also contained remains of adult pollock (mean corrected FL=51.4 ±10 cm, n=192, modal range: 46–52 cm, n_s=81 scats). Percentage overlap based on a comparison of size-frequency distributions totaled 75.1% for those fish eaten around Forrester Island and 52.1% for all fish eaten. However, the estimated overlap would have been assumed incorrectly to be half these values if DCFs had not been applied to the selected digested otoliths and bones (i.e., 36.7% overlap at Forrester and 24.1% for all areas combined). Clearly overlap levels would have been further underestimated if structures in poor condition had been included in our analyses.

Discussion

Only 57% of the scats (303 of 531) that contained suitable pollock remains had structures that were in good enough condition to be measured reliably. Numbers of elements in good or fair condition (n=909) averaged three per scat, and a very small fraction of these consisted of otoliths (<4%). The most numerous structures were DENT, QUAD, and ANGU (Table 1). This finding is inconsistent with feeding trials with captive Steller sea lions where otoliths were found to be the most commonly occurring structure (Cottrell and Trites, 2002; Tollit et al., 2003).

Different structures yielded somewhat different mean sizes of pollock, although 95% confidence intervals generally overlapped, ranging between 37 and 52 cm for bones (Table 2). Such discrepancy is not surprising given that different bones originate from different scats and possibly different fish (even within a single scat). Our comparison of estimates with all structures versus MNI selections indicates that the potential effect of double counting (and measuring) fish within a single scat is likely negligible with large sample sizes (Fig. 2). Although the use of all-structure data to estimate fish length results in a greatly increased sample size, there remains an underlying assumption that all structures are affected equally by digestion. Tollit et al. (2004, this issue) found no significant difference in the degree of erosion across the three size ranges (28.5–45.0 cm FL) for each structure within each condition category.

They also found that the relative shape, structure, and proportion of the morphological features used to estimate erosion were consistent for both smaller and larger fish. We therefore assumed that the DCFs in that study could be used reliably for the fish in our study outside of the experimental size range in which they were considered.

Applying DCFs increased mean fork length estimates by 23% (from 34.4 to 42.4 cm) on average and resulted in adult fish contributing 44% to the sea lion diet by number and 74% by mass. The contribution of juvenile fish was insignificant. Applying valid correction factors clearly provides better insights into prey-size selection and consequently niche overlap. It should also lead to more precise estimates of mass of prey consumed and the number of prey within a scat (Ringrose, 1993; Tollit et al., 1997; Laake et al., 2002).

Over 61 species of prey were identified in the diet of Steller sea lions in Southeast Alaska from 1993 to 1999 (Trites et al.[3]). The most common prey were walleye pollock, Pacific herring (*Clupea pallasi*), Pacific sand lance (*Ammodytes hexapterus*), salmon (*Oncorhynchus* spp.), arrowtooth flounder (*Reinhardtius stomias*), rockfish (*Sebastes* spp.), skates (*Raja* spp.), and cephalopods. During summer, gadids (most of which were pollock) made up 27% of the diet, and increased to 49–62% of the diet at other times of the year (Trites et al.[3]), confirming that pollock are a significant component of the diet.

Steller sea lions consumed a wide size range of pollock in Southeast Alaska; the bulk of fish fell between 20 and 60 cm and peaked between 44 and 52 cm (Fig. 2). The contribution of juvenile fish (≤20 cm) was insignificant. The only historical data to compare with these results are those from the stomach samples of eight Steller sea lions collected from Southeast Alaska in 1986 (Calkins and Goodwin[1]). Pollock lengths backcalculated from all otoliths found in the stomachs were generally shorter (mean FL=25.5 ±10.4 cm, range; 4.8–55.7cm, n=80) than our estimates from multiple structures found in scats collected during the 1990s (mean FL=42.4 ±11.6 cm, range: 10.0–78.1 cm, n=909). It should be noted that we derived our estimates after removing heavily eroded structures and applying DCFs, whereas Calkins and Goodwin[1] did not account for partial digestion. However our estimates of pollock length would have been similar to those of Calkins and Goodwin[1] if we had used only otoliths and had not corrected for digestion (Table 2). Although Frost and Lowry (1980) found no significant difference between the size of otoliths obtained from stomachs and intestines of ribbon seals, underestimates of fish size determined from otoliths from stomach samples will depend on the time since ingestion (i.e., on the extent of digestion).

One possible explanation for the virtual absence of juvenile pollock in the scats we examined is that the relatively smaller structures of smaller fish were more likely to be completely digested, and were therefore underrepresented in the scats (Tollit et al., 1997; Bowen, 2000). However, juvenile pollock otoliths and bones were found in large numbers in a number of scats collected from the western stock (Zeppelin et al., 2004, this issue). Clearly, the potential for underestimating smaller fish depends heavily on the balance between relative recovery rates and the number of different size fish consumed in a meal. For example, if an animal needs to eat 5 kg a day, then it would have to consume 195 15.5-cm pollock, but less than ten 41-cm pollock. Given that large pollock bones are at least three times more likely than small bones to pass through the digestive tract (Tollit et al., 2003; D. J. Tollit, unpubl. data), the sheer numbers of small pollock in this example would lead to a conclusion that smaller fish were more important numerically, when in fact they were equally important. Conversely, the relative proportion of large fish is likely to be overestimated if ten large and ten small pollock are consumed together. The generally low number of structures per scat provides little information to assess this balance. Hence we must assume that our results are representative and unbiased.

Steller sea lions in Southeast Alaska did not seem to eat fish over 65 cm. Whether or not sea lions do not target large fish, or whether large fish are harder to catch and handle, or are encountered at a lower rate is not known. However, large fish could be under-represented in scats if large fish cannot be swallowed whole, and head skeletal parts are lost while the fish is torn apart on the surface (Olesiuk et al., 1990; Wazenbock, 1995) or if bone regurgitation is size specific.

Regional, geographical, and temporal variation in sizes of pollock consumed

Stomach samples collected in 1975–78 and 1985–86 in the Gulf of Alaska contained substantial numbers of juvenile pollock, as well as larger fish (mode: 39–43 cm). In 1985, the distribution of sizes consumed by sea lions around Kodiak Island appeared to mimic that of the pollock population (Merrick and Calkins, 1996). However, juvenile sea lions ate significantly smaller and relatively more juvenile pollock than adult sea lions. Stomachs from the Gulf of Alaska contained an average of 49 pollock (1975–78) and 72 pollock (1985) compared with 1.6 pollock per scat in Southeast Alaska. In the Bering Sea, 90 stomachs were examined between 1975 and 1981 by using only non-eroded otoliths, and these also contained mainly (76%) juvenile pollock (mean FL=29.3 cm), but also some adult fish (Frost and Lowry, 1986).

Between 1998 and 2000, Steller sea lions across the range of the western population in Alaska consumed pollock averaging 39.3 ±14.3 cm (range: 3.7–70.8 cm, Zeppelin et al., 2004, this issue). This finding suggests that sea lions may have been less reliant on juvenile pollock than they were during the 1970s and 1980s. Apparent differences may reflect differences in pollock year-class strength, and thus differences in the dominant size classes that were available to be consumed. However, Zeppelin et al. (2004, this issue) reported that the size distribution of walleye pollock consumed by Steller sea lions between 1998 and 2000 did not appear to fluctuate with year-class strength, unlike the

sizes of Atka mackerel (*Pleurogrammus monopterygius*) consumed in western Alaska.

Comparing samples collected at rookeries from the eastern and western populations reveals that sea lions in the western stock ate significantly greater numbers of smaller pollock and fewer adults in summer than sea lions in Southeast Alaska (Zeppelin et al., 2004, this issue; and our study). However, both eastern and western stock sea lions using haul-outs in March (winter) ate similar size pollock. Adult pollock occurred more frequently in scats collected from rookeries along the open ocean coastline of Southeast Alaska during June and July (74% adults) than they did in scats from haul-outs located in inside waters between October and May (51% adults). Scats collected at rookeries can be considered to be from adult female sea lions and to a lesser extent from adult males, whereas those collected at haul-outs during other times of the year contain a more diverse mix of age groups, including greater numbers of younger sea lions. Thus it is uncertain whether observed size differences in pollock between these two groups are seasonal or due more to size preferences of different aged animals. Limited support for the former comes from the similar size pollock observed in the scats between the two groups in June and July of 1999. Overall, however, it is unknown whether the consumption patterns observed are a result of an actual size selection of prey or if they result from coincidental distributions of sea lions and prey-size classes. Some pinnipeds may select prey of particular sizes (Sinclair et al., 1994) and may encounter difficulties if they cannot switch to other sizes or species if the abundance of preferred prey is reduced. Fine-scale studies are now being undertaken to address such uncertainties.

There are few assessments of pollock stock size for the 1990s in Southeast Alaska (Martin, 1997). However the biomass is believed to have been low compared to other regions of Alaska. Juvenile pollock are known to congregate in the shallow inside waters of Southeast Alaska during winter (Sigler[5]) but are also known to occur in significant numbers in the summer in waters shallower than 200 meters on the outer coastline (Martin, 1997). Recruitment of 1-year-old fish was found to be high during acoustic studies in 1994 and 1999 in the Gulf of Alaska (Guttormsen et al., 2003).

Steller sea lions using rookeries in Southeast Alaska consumed mainly adult pollock between 1994 and 1999 and showed no evidence of tracking any abundant age class of pollock. However, the trend in increasing length estimates for inside haul-outs after 1995 (Fig. 3) does suggest that sea lions might be tracking a particular age class of prey. Certainly a greater range of age classes were consumed at these haul-outs (Fig. 3).

Scientific trawls in 1996 indicated that the larger pollock on the outside coastline occurred generally in waters 201–300 m deep during daylight hours (Martin, 1997) and that smaller pollock were present in shallower depths. Larger pollock tend to disperse and move to shallow waters to feed at night (Smith, 1981). Thus, the observed crepuscular and nighttime foraging by lactating Steller sea lions (Higgins et al., 1988; Trites and Porter, 2002) would be a logical foraging strategy to capture adult pollock. Other important factors, in addition to depth, that likely influence size selection include prey density and spatial distribution in relation to rookeries and haul-outs. Given both the greater mass and energy content of adults compared with juveniles (Perez, 1994; Anthony et al., 2000), the selection of adults would be an energy efficient strategy—all other things being equal.

Overlap in sizes of pollock consumed by Steller sea lions and sizes of pollock caught by fisheries

There was no commercial fishery for pollock in Southeast Alaska during the 1990s. However, a small fishery occurred in nearby Dixon Entrance, B.C., that might indicate sizes that could have been caught in Southeast Alaska if a fishery had occurred. Overlap in sizes of pollock caught by the B.C. fishery with those taken by Steller sea lions further north (our study) more than doubled after applying digestion correction factors (from 24% to 52%). Similarly, high levels of overlap were also found between the sizes of pollock consumed by the western stock (1998–2000) and those caught in the same region by fisheries (after our DCFs were applied to structures recovered from scats—Zeppelin et al., 2004, this issue). A high degree of overlap in size highlights a potential conflict between fisheries and sea lions, but this overlap cannot be considered indicative of competition unless the resource that fisheries and sea lions seek is limited across the space and time in question (Krebs and Davies, 1991).

Conclusions

Our study provides the first substantial description of the size of pollock eaten by Steller sea lions in Southeast Alaska. It also shows the benefits of using bones other than otoliths to estimate the sizes of prey eaten by Steller sea lions, and the importance of correcting for degree of digestion. Accurately reconstructing the sizes of bones and otoliths recovered from scats has a significant bearing, in turn, on accurately determining the mass of prey consumed, and on the extent of overlap of sizes of prey consumed and sizes of the same resource caught in commercial fisheries.

We found that Steller sea lions in Southeast Alaska consumed a large proportion of adult pollock and few juveniles between 1994 and 1999. Although greater proportions of juvenile and adolescent pollock were consumed over the same period, during the summer in the Gulf of Alaska and Bering Sea, larger size fish still were the most abundant prey item in the diet of sea lions. A comparison of these estimates with the lengths of pollock consumed during the 1970s and 1980s shows that

[5] Sigler, M. F. 2003. Unpubl. data. Auke Bay Lab, National Marine Fisheries Service, 11305 Glacier Highway, Juneau, AK 99801.

Steller sea lions can consume a wide range of different size pollock (4–78 cm). Whether or not these differences in sizes of pollock consumed between regions and decades reflect differences in availability, size preferences, or year-class strength is not known and requires further study primarily with fine-scale data from scientific surveys and concurrent scat collections.

Acknowledgments

Funding was provided to the North Pacific Universities Marine Mammal Research Consortium by the National Oceanographic Atmospheric Administration and the North Pacific Marine Science Foundation. We would like to thank the contribution of personnel of the UBC Marine Mammal Research Unit, ADF&G, T. K. Zeppelin, K. A. Call, A. J. Winship, E. H. Sinclair, and two anonymous reviewers. We are also grateful to J. L. Laake and R. Joy for statistical advice.

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Multidirectional movements of sportfish species between an estuarine no-take zone and surrounding waters of the Indian River Lagoon, Florida

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Abstract—We examined movement patterns of sportfish that were tagged in the northern Indian River Lagoon, Florida, between 1990 and 1999 to assess the degree of fish exchange between an estuarine no-take zone (NTZ) and surrounding waters. The tagged fish were from seven species: red drum (*Sciaenops ocellatus*); black drum (*Pogonias cromis*); sheepshead (*Archosargus probatocephalus*); common snook (*Centropomus undecimalis*); spotted seatrout (*Cynoscion nebulosus*); bull shark (*Carcharhinus leucas*); and crevalle jack (*Caranx hippos*). A total of 403 tagged fish were recaptured during the study period, including 65 individuals that emigrated from the NTZ and 16 individuals that immigrated into the NTZ from surrounding waters of the lagoon. Migration distances between the original tagging location and the sites where emigrating fish were recaptured were from 0 to 150 km, and these migration distances appeared to be influenced by the proximity of the NTZ to spawning areas or other habitats that are important to specific life-history stages of individual species. Fish that immigrated into the NTZ moved distances ranging from approximately 10 to 75 km. Recapture rates for sportfish species that migrated across the NTZ boundary suggested that more individuals may move into the protected habitats than move out. These data demonstrated that although this estuarine no-take reserve can protect species from fishing, it may also serve to extract exploitable individuals from surrounding fisheries; therefore, if the no-take reserve does function to replenish surrounding fisheries, then increased egg production and larval export may be more important mechanisms of replenishment than the spillover of excess adults from the reserve into fishable areas.

Manuscript submitted 12 May 2003 to Scientific Editor's Office.
Manuscript approved for publication 20 January 2004 by the Scientific Editor.
Fish. Bull 102:533–544 (2004).

Fishery reserves or no-take sanctuaries, defined as areas where all fishing activities are prohibited, are increasingly proposed as an additional measure to traditional fishery management practices for protecting fish populations from overexploitation (PDT, 1990; Bohnsack and Ault, 1996). The American Fisheries Society recently issued a policy statement on the protection of marine fish stocks at risk of extinction and supported the development of large marine reserves to protect and rebuild vulnerable populations (Musick et al., 2000). Although reserves have been established primarily in reef or coastal marine habitats, the potential to apply similar management strategies in estuarine systems may also be possible (Johnson et al., 1999; Roberts et al., 2001).

Reserves in estuarine areas may help protect exploitable fishery species. Increases in species' sizes and densities within these reserves may also enhance adjacent fisheries by two separate mechanisms. Johnson et al. (1999) found that an existing estuarine no-take sanctuary on Florida's central east coast protected populations of larger, spawning-age sportfish species. As a result, they suggested that protection of populations in no-take sanctuaries could also lead to the replenishment of surrounding fisheries through increased egg production, larval export, and juvenile recruitment. Additionally, mark-recapture data have demonstrated that large juvenile and adult fishes emigrate from estuarine protected areas to surrounding waters (Bryant et al., 1989; Funicelli et al., 1989; Johnson et al., 1999; Roberts et al., 2001; Stevens and Sulak, 2001) and these data have been used to suggest that spillover of excess adult fish from estuarine reserve areas can directly supplement nearby fisheries. Roberts et al. (2001) concluded that the abundance of International Game Fish Association based on line-class-record catches in the vicinity of the estuarine no-take sanctuary on Florida's east coast resulted indirectly from protection and spillover of large adults to outlying waters.

It has also been suggested that reserves protect areas of undisturbed habitat (PDT, 1990), either by design or through cessation of destructive practices, and reserves are commonly established in areas of pristine, productive, or otherwise important habitats required by the species being protected (e.g., Russ, 1985). Furthermore, studies have shown that protecting fishery species can indirectly change the overall community structure (Cole and Keuskamp, 1998) and, under certain circumstances, can increase primary and secondary productivity (Sala and Zabala, 1996; Babcock et al., 1999). The influence of habitat quality on fish movements in relation to protected areas has not been investigated; however, reserve habitats that offer potential advantages in the form of improved habitat quality (Chapman and Kramer, 1999)

or increased food and habitat availability could be expected to attract, or at least retain, individuals that immigrate to the reserves from surrounding unprotected habitats. Reserve areas that attract and retain exploitable individuals from surrounding habitats at higher rates than they replenish the surrounding habitats could be considered to be sinks in terms of their ability to directly supplement adjacent fisheries through spillover of exploitable-size individuals. Fish emigration from reserve habitats and the replenishment of nearby fisheries is a commonly predicted benefit of harvest reserves (see reviews in Roberts and Polunin, 1991, and Rowley, 1994). However, there are currently no studies that simultaneously examine emigration and immigration in relation to estuarine reserves or that document the extent to which reserve areas may also function to withdraw individuals from surrounding fisheries. Without an assessment of net exchange, the interpretation of reserve benefits with respect to replenishment cannot be properly evaluated.

The National Aeronautics and Space Administration (NASA) closed a portion of the Indian River Lagoon at the Merritt Island National Wildlife Refuge (MINWR) on Florida's east coast for security purposes in 1962. A direct result of this closure was the effective creation of an estuarine no-take zone that remains to the present time. The proximity of this no-take zone to productive estuarine fisheries provided an opportunity to examine sportfish movements in the area with mark-recapture methods. Johnson et al. (1999) first documented sportfish migrations out of this no-take sanctuary, and in a related study, Stevens and Sulak (2001) provided more complete descriptions of movement patterns of individual species; each of these studies provided evidence that the restricted habitats protected fish populations and that adult sportfish egressed into surrounding waters open to fishing. However, because all tagged fish originated from within restricted habitats, in neither of these studies was it possible to consider the potential for the movements of fish into protected areas from surrounding waters. Therefore, we (sponsored by the Florida Fish and Wildlife Conservation Commission-Florida Marine Research Institute [hereafter referred to as FMRI] Fisheries-Independent Monitoring Program) tagged fish species throughout the northern Indian River Lagoon system, including both the MINWR no-take zone and the surrounding lagoon waters, from 1990 to 1999. We investigated the relationship between sportfish egress and ingress in relation to the MINWR no-take zone and offer a quantitative foundation for the discussion of net fish movements into or away from protected estuarine habitats.

Materials and methods

Study area

The Indian River Lagoon (IRL) is a shallow barrier island estuarine system spanning 253 km along the central east coast of Florida between Ponce de Leon Inlet in Volusia County and Jupiter Inlet in Palm Beach County. The lagoon is composed of three relatively isolated basins: Mosquito Lagoon, the Indian River proper, and the Banana River (Fig. 1). These three basins maintain hydrological connections with each other through narrow man-made channels at Haulover Canal and the Merritt Island Barge Canal (shown on Fig. 2) and through a natural channel at the southern end of the Banana River. Hydrodynamic exchange and fish passage between the lagoon and the Atlantic Ocean occur primarily through five inlets, which are concentrated in the southern half of the system. The hydraulic lock system located at Port Canaveral provides only an intermittent opportunity for exchange between the IRL and Atlantic Ocean. Gilmore et al. (1981) and Mulligan and Snelson (1983) have provided detailed descriptions of the lagoon and its habitats.

The no-take zone (NTZ) created by NASA and MINWR is located at the northern terminus of the Banana River basin of the lagoon. An earthen causeway defines the southern boundary of this no-access security area and contains only two openings that permit fish to migrate to and from adjacent waters. Much of the natural shoreline and saltmarsh habitats in the lagoon have been altered for mosquito control purposes. However, actual shoreline habitats surrounding MINWR—including the NTZ, the northern Banana River basin, the northern Indian River basin, and Mosquito Lagoon—remain relatively undeveloped in comparison to the urban shoreline development in the southern IRL. Detailed descriptions of the habitat composition within the NTZ and surrounding study area were provided by Johnson et al. (1999).

Data collection

Fish were tagged as part of several related FMRI projects (stratified-random, fixed-station, and directed sampling designs) in the northern IRL between 1990 and 1999 (FMRI[1]). In most cases, tagging was conducted opportunistically on healthy fish following capture in multipanel monofilament gill nets, nylon trammel nets, nylon haul seines, or on hook and line. In other cases, projects were designed specifically to assess tag-recapture information (Murphy et al., 1998). Because of the focus of our sampling programs in this area, the majority of our tagging efforts occurred north of Sebastian Inlet within the Indian and Banana River basins of the lagoon. A small percentage of tags were placed in fish captured south of Sebastian Inlet or in Mosquito Lagoon. Overall, our sampling collections in the NTZ

[1] FMRI (Florida Fish and Wildlife Conservation Commission). 1999. Florida Marine Research Institute, Fisheries-independent monitoring program, 1999 annual data summary report. In-house Report, Florida Fish and Wildlife Conservation Commission, Florida Marine Research Institute, 100 Eighth Ave. S.E., St. Petersburg, Florida, 33701.

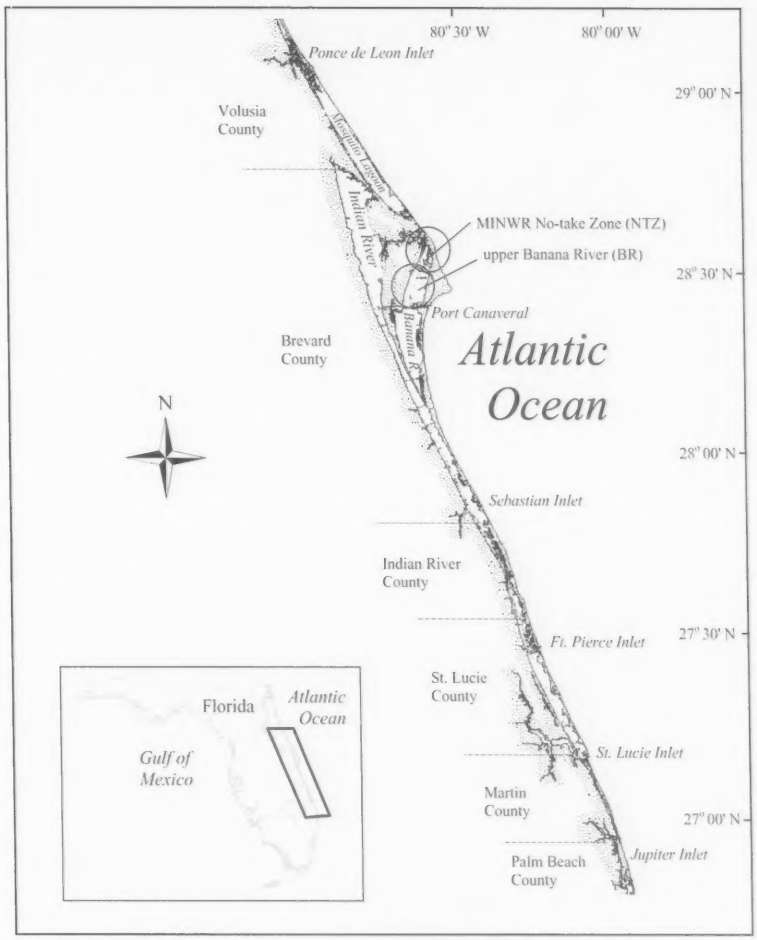

Figure 1
Map of Florida and the Indian River Lagoon study area.

accounted for approximately 20% of our total sampling efforts and averaged approximately 1–2 days/month over the study period.

Fish were tagged by inserting 50-mm, 70-mm, or 100-mm Hallprint dart tags (Halprint Ltd., Victor Harbor, South Australia) into the dorsal musculature; the plastic dart was lodged beneath the pterygiophores of the dorsal fin. Each tag contained a visible external streamer with a unique alphanumeric code and instructions for anglers to contact us with recapture information in order to collect a reward (five dollars or equivalent). Information recorded at the time of initial tagging included the tag number, species tagged, date, location (latitude and longitude), and fish length (standard, fork, and total lengths as appropriate for the species). Recapture information on tagged fish was collected through August 2000 from angler reports and from fish recaptured during FMRI sampling activities. Because of public-access prohibitions, recapture information from inside the MINWR NTZ was gathered exclusively through FMRI sampling efforts. Data requested for recaptured fish included the same information as that recorded at initial tagging; however, in several cases, length or precise location information returned from anglers was considered to be unreliable, which prevented accurate statistical comparisons of relationships involving recapture lengths or distances traveled. Therefore, reported length data are limited to initial tagging information only (total length; TL). To prevent problems with pseudoreplication for individuals recaptured on multiple occasions, we included only the initial tag recovery data in our calculations of recapture percentages.

Overall patterns of fish migrations, including general recapture locations and direction of movements into or away from the NTZ, were described by using data

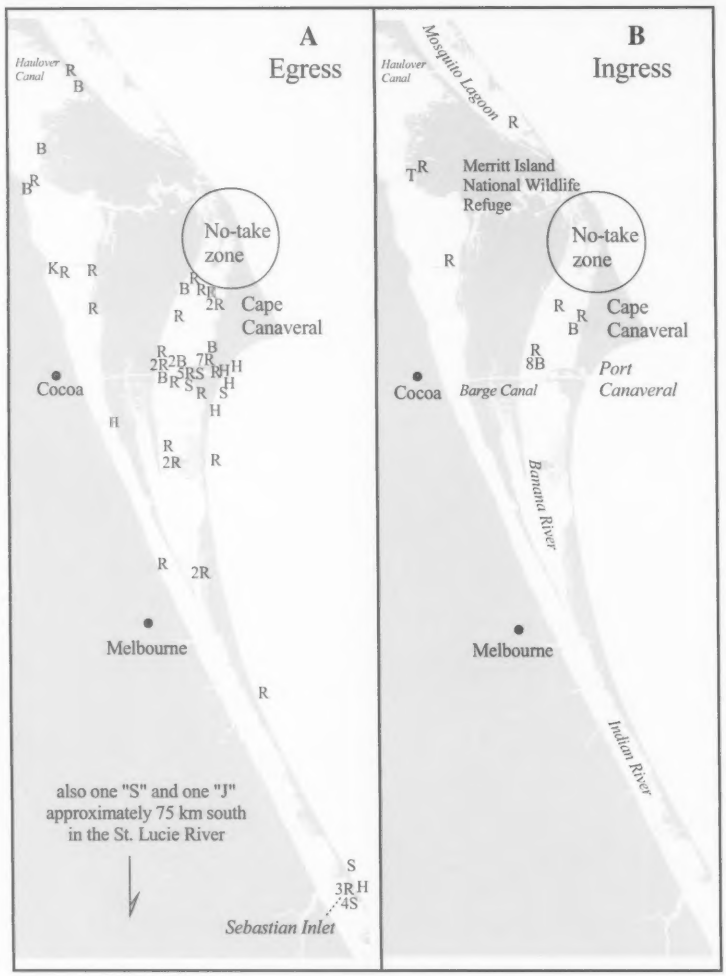

Figure 2

(**A**) Recapture locations of tagged fish that migrated out of the Merritt Island National Wildlife Refuge no-take zone. (**B**) Original tagging locations of fish that migrated into the Merritt Island National Wildlife Refuge no-take zone. R = red drum, B = black drum, S = common snook, H = sheepshead, T = spotted seatrout, J = crevalle jack, K = bull shark. Numbers before species codes (letters) indicate the number of individuals of that species that were captured at that location.

from all available recapture sources. In contrast, we calculated migration rates exclusively from the recapture data collected during FMRI sampling activities. Although this procedure excluded tag-return data from recreational anglers, it permitted a quantitative assessment of recapture rates based on standardized FMRI collection gear, comparable sampling effort, and 100% tag reporting rates. We resolved potential problems related to differences in habitat characteristics and sampling intensity by including only data from the NTZ and a fishable area of a similar size and habitat type in the adjacent Banana River (BR, Fig. 1). This BR zone corresponded precisely to the sampling zone used for population comparisons in Johnson et al. (1999), denoted as "FBR" (fished Banana River) in that study. Species that did not contribute any FMRI recapture information in either of these two areas were excluded from our analyses. Tag recovery and migration rates were calculated separately for the NTZ and BR. For our purposes, "migration" was defined as a directional fish movement across the NTZ boundary from the original tagging location, and we made the assumption that the migration patterns of recaptured fish represented the migration patterns of the overall population. Rela-

Table 1

Summary of tagging and recapture data for seven of the most common sportfish species tagged by FMRI scientists in the northern Indian River Lagoon study area. Locations where tag and recapture data were collected are separated into the no-take zone (NTZ) and the surrounding waters of the Indian River Lagoon (IRL).

	No-take zone			Indian River Lagoon				
		Recapture location			Recapture location			
Species	Tagged inside NTZ	NTZ	IRL	Tagged outside NTZ	NTZ	IRL	Total no. of fish tagged	Total no. recaptured and percent recaptured
Bull shark (*Carcharhinus leucas*)	1		1	24		1	25	2 (8.0)
Common snook (*Centropomus undecimalis*)	104	1	9	406		32	510	42 (8.2)
Crevalle jack (*Caranx hippos*)	55		1	59		1	114	2 (1.8)
Sheepshead (*Archosargus probatocephalus*)	597		6	520		26	1117	32 (2.9)
Spotted seatrout (*Cynoscion nebulosus*)	193	2		171	1	3	364	6 (1.6)
Black drum (*Pogonias cromis*)	637	4	8	831	9	32	1468	53 (3.6)
Red drum (*Sciaenops ocellatus*)	720	30	40	1344	6	190	2064	266 (12.9)
Total	2307	37	65	3355	16	285	5662	403 (7.1)

tive migration rates were calculated as the percentage of recaptured fish that migrated from their original tagging location. These migration rates and their reciprocal (retention rates) were compared between the NTZ and the BR to determine the relative potential for sportfish movements into or away from protected habitats. Chi-square contingency tests for frequency data (with Yates's correction for small sample sizes) were used to test the hypothesis that recapture location was independent of the tagging location.

Results

A total of 5951 fish of 27 species were tagged during FMRI sampling within the IRL between September 1990 and December 1999. However, because 95% of these fish were represented by only seven species (Table 1), which included all fish that migrated across the reserve boundaries, only these seven species were considered further in our analyses. Red drum (*Sciaenops ocellatus*) was the most commonly tagged species (n=2064), followed by black drum (*Pogonias cromis*, n=1468), sheepshead (*Archosargus probatocephalus*, n=1117), common snook, (*Centropomus undecimalis*, n=510), spotted seatrout (*Cynoscion nebulosus*, n=364), crevalle jack (*Caranx hippos*, n=114), and bull shark (*Carcharhinus leucas*, n=25). Approximately 41% (n=2307) of these fish were tagged inside the boundaries of the NTZ. The remainder (n=3355) were tagged in the surrounding lagoon. Through August 2000, 403 tagged fish (7.1% of total) were recaptured and reported either by FMRI staff sampling in the lagoon or by the public. Overall recapture rates were highest for red drum (12.9%), followed by those for common snook (8.2%), bull shark, (8.0%), black drum (3.6%), and sheepshead (3.0%).

Tagged fish were generally representative of the larger mobile members of the species and encompassed the legally exploitable size ranges for species with management restrictions (Table 2). For species except the bull shark and red drum, mean lengths of fish tagged inside the NTZ exceeded those of fish tagged outside the NTZ.

Approximately 25% (n=102) of the 403 total recaptured fish were fish originally tagged inside the NTZ (Table 1). Thirty-seven of these fish were also recovered inside the NTZ, including three red drum that were subsequently recaptured on multiple occasions in the protected area. The remaining 65 recaptured fish were caught after emigrating to outlying waters, including one red drum that was recaptured a second time outside the NTZ. Species that migrated out of the NTZ were red drum (n=40, mean TL=643 mm, SD=135 mm), common snook (n=9, mean TL=570 mm, SD=97 mm), black drum (n=8, mean TL=845 mm, SD=88 mm), sheepshead (n=6, mean TL=398 mm, SD=38 mm), bull shark (n=1, TL=789 mm), and crevalle jack (n=1, TL=628 mm). Recapture distances ranged from 0 km immediately outside the NTZ to approximately 150 km south in the St. Lucie River estuary, but recaptured fish were more abundant closer to the NTZ (Fig. 2A). Most of the recaptured fish were concentrated in areas of high fishing pressure, such as causeways, inlets, and waters near the boundary of the NTZ. Collectively, fish that emigrated from the NTZ did not appear to show a bias for any one direction of movement: recaptured fish were found both northward in the Indian River and southward throughout both the Indian River and Banana River basins of the lagoon. For individual species, red drum that emigrated were distributed throughout the lagoon system and coastal habitats, whereas black drum were predominantly recaptured in the northern

Table 2

Total length (TL) size ranges (in mm) and legal size limits (as of August 2000) for tagged sportfish species from the no-take zone and the outlying Indian River Lagoon study area.

	No-take zone			Indian River Lagoon			Legal size limits
Species	Mean (SD)	min	max	Mean (SD)	min	max	(mm TL)
Bull shark	789 (—)	789	789	974 (135)	684	1180	None
Common snook	570 (106)	330	844	506 (138)	227	944	660–864 (+ 1 over)
Crevalle jack	486 (140)	305	720	443 (113)	264	720	None
Sheepshead	398 (68)	235	614	365 (76)	171	594	305 minimum
Spotted seatrout	415 (129)	185	754	335 (111)	212	678	381–508 (+ 1 over)
Black drum	786 (129)	249	1156	742 (240)	225	1135	356–610 (+1 over)
Red drum	613 (166)	308	1245	624 (229)	203	1210	457–686

estuarine portion of the study area. Sheepshead and common snook were recaptured primarily to the south at inlets or in the adjacent Atlantic coastal waters outside the lagoon.

The remaining 75% (n=301) of the total recaptured fish were from fish originally tagged outside the NTZ (Table 1). The majority of these (n=285) were also recovered in outlying waters, including 16 red drum and 1 sheepshead that were subsequently recaptured on multiple occasions. Sixteen fish were recaptured after they had immigrated into the reserve. These recaptured fish were from three sciaenid species: predominantly black drum (n=9, mean TL=907 mm, SD=66 mm) and red drum (n=6, mean TL=656 mm, SD=170 mm), but also one spotted seatrout (TL=420 mm)(Fig. 2B). The longest migration distances into the NTZ were up to 75 km for red drum and spotted seatrout tagged in southern Mosquito Lagoon and the northern Indian River basins. All black drum that immigrated into the NTZ were tagged in the adjacent Banana River basin.

A relatively large number of red drum, common snook, and sheepshead that were tagged inside the NTZ or in the outlying waters were recaptured in close proximity (0 to 2.75 km distance) to inlet habitats. Recaptured red drum from inlet habitats (n=45, mean TL=647 mm, SD=135 mm) peaked during September through November. Recaptured common snook from inlet habitats (n=13, mean TL=598 mm, SD=111 mm) were distributed throughout much of the year but peaked in late fall. Few common snook were recaptured from inlet spawning habitats during the peak summer spawning months (June–August) when their fishery was closed. Recaptured sheepshead from inlet habitats (n=8, mean TL=373 mm, SD=53 mm) were concentrated in the winter and early spring.

Estimated migration rates were calculated by using only those fish that were tagged and recovered from FMRI sampling in the NTZ and the immediately adjacent upper Banana River (BR). The number of fish tagged in the NTZ (n=1654) was approximately 1.7 times the number tagged in the BR (n=965) (Table 3); however, the overall recapture rates of fish that were originally tagged in each of these two areas were equal (2.4%). Black drum and red drum made up the majority of tagged and recaptured fish in both areas and were the only species recaptured that had migrated both into and away from the NTZ in this comparison. For total sportfish (all species pooled), there was a significant relationship between the tagging location and the direction of fish movements ($\chi^2_{1,\ 0.05}$=13.8, P=0.0002). A total of 40 fish originating from the NTZ were recaptured, but that number included only 2 fish (one red drum and one black drum) that emigrated to the BR (5% overall migration rate). In contrast, 23 fish originating in the BR were recaptured overall, including 12 that immigrated into the NTZ (52% overall migration rate). Species-specific migration rates were highest for black drum, and relative immigration rates (90%) were higher than emigration rates (25%). For this species, the frequency of immigration and emigration were statistically independent of tagging location ($\chi^2_{1,\ 0.05}$=0.01, P=0.9039), which is probably due to the low number of recaptures of fish tagged inside the NTZ (Table 3). For red drum, relative immigration rates (27%) were also higher than emigration rates (3%), but in this case, there was a significant relationship between fish movements and tagging location ($\chi^2_{1,\ 0.05}$=20.58, P<0.0001). Common snook, spotted seatrout, and sheepshead were also recaptured by FMRI scientists in these comparisons, but none of these recaptured fish represented evidence of migrations across the NTZ boundary from their original tagging location.

Discussion

This study demonstrated both the emigration and immigration of sportfish species across the boundaries of an estuarine no-take zone (NTZ). Legal-size large juveniles and adults of six of the recreationally valuable species tagged within NTZ boundaries—red drum, black drum, common snook, sheepshead, bull shark, and crevalle

Table 3

Summary of tag and recapture data from only the Florida Marine Research Institute sampling efforts in the no-take zone (NTZ) and the adjacent fished waters of the Banana River (BR). Species that did not contribute any recapture information were not included in calculations of totals or of migration percentages.

	No-take zone					Banana River				
		No. fish recaptured					No. fish recaptured			
	No. of fish tagged	NTZ	BR	Total	Percent that migrated	No. of fish tagged	NTZ	BR	Total	Percent that migrated
Red drum	720	32	1	33	3.3	176	3	8	11	27.3
Black drum	637	3	1	4	25.0	495	9	1	10	90.0
Common snook	104	1	0	1	0	62	0	1	1	0.0
Spotted seatrout	193	2	0	2	0	121	0	0	0	—
Sheepshead	597	0	0	0	—	232	0	1	1	0.0
Totals	1654	38	2	40	5.0	965	12	11	23	52.2
Tag recovery (%)				2.4					2.4	

jack—were documented to migrate out of the protected area. Johnson et al. (1999) and Stevens and Sulak (2001) also observed many of these same species emigrating from no-take zones within the same refuge system during the late 1980s, although the species with the highest recapture rates in their studies (common snook) differed from the current study (red drum). This difference may reflect an increase in the popularity of the red drum fishery on Florida's east coast during the current study period. Since 1989, when the recreational red drum fishery reopened under strict management regulations, there has been a significant increase in both the total red drum landings on the Atlantic coast and in the estimated number of fishing trips made by anglers seeking or catching red drum each year (Murphy[2]). Tagging studies in estuarine areas of the Everglades National Park have previously documented emigrations of striped mullet (*Mugil cephalus*), gray snapper (*Lutjanus griseus*), and spotted seatrout away from protected habitats (Bryant et al., 1989; Funicelli et al., 1989). Recent studies suggest that fish moving out of protected areas in the IRL may help to replenish nearby fisheries and may contribute to trophy fisheries in the surrounding system (Johnson et al., 1999; Roberts et al., 2001).

In our study, overall emigration rates were low, but many of the fish that emigrated from the estuarine NTZ moved comparatively large distances. The egress patterns of exploitable species may affect both the species' potential for protection and the degree to which fisheries located adjacent to protected reserves will be enhanced (DeMartini, 1993). In coastal marine and tropical reef systems, where the large majority of reserves have been established, long-distance movements greater than a few kilometers by demersal fishery species are limited to a very small percentage of individuals (Beaumariage, 1969; PDT, 1990 and references therein; Rowley, 1994), and the direct supplementation of nearby fisheries by exploitable species appears to be highly localized (Buxton and Allen, 1989; Russ and Alcala, 1996). The majority of fish that emigrated from the NTZ were recaptured between 10 and 75 km from the boundary, but fish were also recovered as far as 150 km from the NTZ boundary. Our observations on migration distances and recapture locations corresponded well with those reported from previous studies of fish movements out of this same reserve system (Johnson et al., 1999; Stevens and Sulak, 2001), although maximum recapture distances in earlier studies were even greater.

Many of the fish that emigrated from the NTZ—such as red drum, common snook, and sheepshead—were recaptured at inlet locations or in the nearshore coastal waters at sizes that were large enough to include reproductively mature adults (Murphy and Taylor, 1990; Render and Wilson, 1992; Taylor et al., 2000). The seasonality of inlet-associated recaptures was consistent with the seasonality of documented spawning and movement patterns for these species. In Florida, red drum typically spawn in nearshore coastal waters during the fall (Murphy and Taylor, 1990), although spawning within the IRL has also been documented (Johnson and Funicelli, 1991). Spawning by common snook may occur year-round on Florida's east coast (Gilmore et al., 1983), but most spawning takes place between May and October in or near major inlets to the Atlantic Ocean (Taylor et al., 1998). The limited number of common snook recaptured from inlet spawning habitats during the peak summer spawning season (June–August) was likely due to the fishery being closed during those months. Sheepshead move offshore with the onset of cool weather in the late fall (Gunter, 1945; Kelly, 1965), and spawning likely occurs in offshore waters during

[2] Murphy, M. D. 2002. A stock assessment of red drum, *Sciaenops ocellatus*, in Florida: status of the stocks through 2000, 32 p. Florida Fish and Wildlife Conservation Commission Report, Melbourne, FL.

the spring (Springer and Woodburn, 1960; Jennings, 1985; Tucker and Barbera, 1987). In the northern portion of the IRL, where the NTZ is located, the closest access to the coastal environment is through two inlets located approximately 75 km (Sebastian Inlet) and 100 km (Ponce de Leon Inlet) swimming distance away or through an intermittent lock opening at Port Canaveral approximately 12 km to the south. In order to reach nearshore or tidal-pass spawning habitats, species must first migrate to these locations. The coincidence of tag recoveries from these areas during identified spawning or migration periods likely indicated that the relatively long movement distances we observed resulted from a combination of geographical, environmental, and biological factors, including the proximity of the NTZ to habitats that are important for specific life-history requirements of individual species. From a management viewpoint, these relationships can affect the spatial extent of species' migrations in relation to protected habitats, as well as the degree of protection provided to individuals that are migratory, and should be considered carefully in the design of estuarine reserves.

This study documented the ingress of exploitable estuarine sportfish species into protected habitats and demonstrated that these movements can also cover substantial distances. Species moving towards the NTZ traveled distances of at least 10–75 km. The original tagging locations of these fish were distributed throughout the northern Indian and Banana rivers and southern Mosquito Lagoon, which paralleled the primary region of our tagging efforts. Whether or not fish from more southerly locations in the IRL system would migrate into the NTZ is largely unknown because of the lack of tagging effort in those areas. However, for tropical species such as the common snook, permit (*Trachinotus falcatus*), gray snapper, and others whose abundances increase seasonally in the northern lagoon habitats during the warmer months (Tremain and Adams, 1995), it seems probable that seasonal movements could bring them into contact with the protected habitats. In such cases, these species would benefit only temporarily from fishing protection until their return migrations made them again vulnerable to capture. In contrast, species observed migrating into the NTZ that typically have a high degree of site fidelity during specific life-history stages, such as the red drum (Beaumariage, 1969; Adams and Tremain, 2000), black drum (Murphy et al., 1998), and spotted seatrout (Moffett, 1961), should derive greater long-term benefits from reserve protection following immigration into protected areas.

Tagging studies that examine the transfer of fishery species between reserve and outlying habitats are rare, and we have found only one recent study on any fishery species, the American lobster (*Homarus americanus*), that investigated the effects that multidirectional species migrations may have upon protective reserve functions (Rowe, 2001). Studies in which fish movements have been examined, in both estuarine and marine protected areas, have focused exclusively on fish egress from reserve habitats (Bryant et al., 1989; Buxton and Allen, 1989; Funicelli et al., 1989; Holland et al., 1996; Zeller and Russ, 1998; Johnson et al., 1999, Stevens and Sulak, 2001) or on home ranges of species associated with reserve habitats (Eristhee and Oxenford, 2001; Starr et al., 2002). In the present study, we simultaneously examined both egress and ingress of sportfish in relation to a no-take reserve and the surrounding unprotected waters, and the results provide a starting point to quantitatively discuss the relationship between fish emigration and immigration, as well as the implications of such movements to the resulting functions of replenishment to or withdrawal from nearby estuarine fisheries. When all recapture sources were considered, the ratio of migrating to nonmigrating individuals was much higher for fish tagged inside the NTZ (1.58) than for those tagged outside the NTZ (0.05); this ratio implies that there is a spillover effect from the reserve. However, this difference is less apparent when measured against the large disparity between recapture effort from inside the NTZ (12–24 FMRI sampling days/year + 12–24 angler days/year) and recapture effort from the surrounding lagoon waters of Brevard County (50–100 FMRI sampling days/year + 114,000–181,000 angler days/year [FMRI, unpubl. data]). Furthermore, this direct comparison assumes that recapture potential was the same in protected and unprotected areas, which is unlikely given the differences between the primary recapture gear used in scientific research activities inside the reserve (nets) and the gear used in recreational angling outside the reserve (hook and line). There were no reliable estimates of sportfish species landings available for the limited study region that could have enabled us to intercalibrate for these differences; therefore, we limited further comparisons to only data recovered through FMRI sampling activities in the northern Banana River basin. This limitation came at the expense of important tag-recovery data collected by anglers or collected from more outlying areas of the lagoon but permitted a more quantitative comparison of migration potential that focused comparisons on immediately adjacent areas where the effects of spillover would most likely be realized (Buxton and Allen, 1989; Russ and Alcala, 1996). In these comparisons, a disproportionate number of fish were tagged inside the NTZ, but overall tag-recovery rates for fish originating in both the NTZ and the adjacent Banana River were equivalent. This finding indicated that tagged individuals from both areas were equally susceptible to recapture. However, there were substantial differences in the migration patterns of fish between the two areas. In the vicinity of the NTZ, the relative potential for overall sportfish migrations (primarily for red drum and black drum, which provided the greatest quantity of tag recovery data) towards the NTZ from unprotected habitats (52%) was greater than the potential for migrations out of the NTZ (5%).

Two potential limitations must be considered when comparing these migration rates. First, it is possible that recreational fishing in the upper Banana River could have reduced the number of tags available to FM-

RI sampling activities outside the NTZ, leading to lower tag recovery rates from this area. However, several fish from the Banana River study area were recaptured on multiple occasions—a common occurrence in this region where fish are caught and released in fishing practices. Although there is some postrelease cryptic mortality associated with catch-and-release practices, these releases likely limited the effects of local fishing on our analyses. Second, our assumption that the migration patterns of recaptured fish represented the migration patterns of the overall population may not be valid if the respective length frequencies were not also equally represented. The use of multiple gear types and sampling strategies to collect fish for tagging increased the likelihood that the length frequencies of species in our collections represented the available population. Reported recapture length frequencies closely approximated the population length frequencies in our collections for red drum, black drum, and sheepshead but over-represented the frequency of larger individuals for common snook and spotted seatrout. Because red drum and black drum were the principal species that displayed multidirectional migration patterns, we considered the potential for size bias to be minimal in our comparisons of estimated ingress and egress rates.

Ultimately, a determination of the net result of these migration patterns, in terms of replenishment to or withdrawal from adjacent fisheries, would require accurate assessments of species population abundances that were beyond the scope of this study. If there are large enough differences in population densities across the NTZ boundary, either as a result of increased production inside the reserve or high fishing mortality outside, then the relatively low emigration rates that we observed could still result in a net export of exploitable individuals to fished populations in surrounding waters. In trammel-net collections from this same reserve during the late 1980's, Johnson et al. (1999) estimated that in the protected habitats, relative abundances of red drum populations were 6.3 times greater and of black drum were 12.8 times greater than the relative abundances of these populations in adjacent unprotected areas. More recent shoreline haul-seine data from 1997–2000 show that these abundances were only 1.8 times greater for red drum and 1.5 times greater for black drum (FMRI, unpubl. data). To what extent the difference in abundance estimates between these two temporally separate studies is related to fish movements, to stringent changes in management regulations that have occurred, or to the difference in sampling methods used is undetermined. However, if we consider the more recent population level differences between the NTZ and adjacent waters, then the emigration and immigration rates observed in the present study indicate that there is a potential for more substantial movements by these species towards protected habitats than away from them.

One limitation of tag-recapture data is that such data provide only a snapshot view of overall fish movements, and the whereabouts of tagged individuals between the time of tagging and recapture are unknown. It is possible that the movements we observed for red drum and black drum in the vicinity of the NTZ were simply instantaneous views of a more complex series of movements between the NTZ and adjacent waters. One possibility is that these movements could be related to daily or seasonal home ranges that extend across reserve boundaries. Studies that attempt to quantify home ranges for these species at any temporal scale are limited. Carr and Chaney (1976) followed a single red drum, which was fitted with an ultrasonic transmitter, for up to two days after releasing it into the Intracoastal Waterway near St. Augustine, Florida. During that time, fish movements were oriented against the direction of tidal flow but remained within 2 km of the release point. Adams and Tremain (2000) found that large juvenile red drum repeatedly used or were continually associated with a 2-km section of a northern IRL tidal creek for periods of up to 18 months. Tagging studies from estuarine waters generally indicate that the majority of red drum and black drum do not make substantial movements from their release sites, although some individuals are capable of migrating up to several hundred kilometers (Beaumariage, 1969; Osburn et al. 1982; Music and Pafford, 1984; Murphy et al., 1998). During the present study, 20 red drum were recaptured on multiple occasions; however, none of these fish exhibited movements that could provide evidence for home ranges that overlapped the NTZ boundaries. Another possibility for the movement patterns we observed is that they are related to population equilibrium adjustments that occur when the relative attributes of the NTZ and surrounding areas change with respect to each other. For example, beginning in 1990 and coinciding with the onset of the present study, the Banana River adjacent to the NTZ (including much of our BR study area) was closed to motorized boat traffic. Although the area remained open to fishing, it became considerably more difficult to access by fishermen. If this limitation resulted in lower fishing pressure (i.e., predation) and fewer habitat disturbances, then the relative habitat value and rates of migration into this area may have increased during that time. There are no quantifiable estimates of migration rates prior to this study for comparison, but our results do not demonstrate an equilibrium adjustment toward potentially higher quality BR habitats during our study period. If species movements are not equilibrium adjustments, but rather are driven by an attraction to or retention within habitats that offer protective benefits, then ultimately reserve habitats should become saturated. Predicted equilibrium population sizes for queen conch (*Strombus gigas*) and spiny lobster (*Panulirus argus*) were achieved in just three years after the effective creation of a Caribbean reef harvest refuge, but models suggested that relatively minor changes in refuge area and boundary condition (i.e., permeability) could result in major population-level responses by exploited species, depending upon dispersal dynamics and habitat availability (Acosta, 2002). The estuarine no-take zone at

MINWR has been in effect for approximately 40 years, presumably long enough for fish populations to reach equilibrium levels, yet we observed a net movement of fish into protected habitats over the past decade.

A wide range of factors interact to determine the distributions of large mobile fish in the IRL, where physical environmental conditions (salinity, inlet distance, temperature, etc.) have a primary influence on the species' distributions over a lagoon-wide scale, and where species responses to biological variables (seagrass cover, depth, seasonality, etc.) act secondarily to influence distributions at smaller scales (Kupschus and Tremain, 2001). The specific mechanisms that lead to the greater ingress rates into the NTZ for red drum and black drum in the present study cannot be determined from our data. Possibilities include a behavioral attraction to the NTZ due to the interrelated influences of habitat preference, spawning, and social structure, or due to potentially higher retention rates after migration into the reserve. Red drum and black drum were routinely observed foraging in large schools within both the NTZ and surrounding waters, which suggested that food resources were available in each of these habitats; however, there are few studies that have attempted to quantify differences in resource availability between these areas. Johnson et al. (1999) described the habitat characteristics of their study areas within the same reserve system but found that protection from fishing, and not habitat difference, was the primary factor contributing to differences in the abundance of sportfish species between fished and unfished areas. The availability of suitable spawning habitats within the NTZ may also attract red drum and black drum to the reserve habitats. We observed indications of reproductive behavior by both of these species inside the NTZ that is common among members of the drum family, including concentrations of drumming fish (Mok and Gilmore, 1983) and repeated side-to-side contact among individual fish (Tabb, 1966) in the presence of ripe and running males. Although we did not directly observe these behaviors for either species outside of the NTZ, black drum and red drum are documented to spawn elsewhere within the IRL system (Mok and Gilmore, 1983; Johnson and Funicelli, 1991) and we cannot automatically presume that suitable spawning habitats do not also occur in the surrounding waters. If there is a behavioral attraction to protected habitats, then the subsequent retention of individuals that have immigrated into these areas may be prolonged by the limited boundary permeability of this reserve, which contains only two potential egress pathways back into the adjacent waters. In order to fully understand the protective functions of this estuarine reserve and others, it will be important to identify the biological, behavioral, and physical mechanisms that influence species movements in relation to the reserve boundaries.

The opportunistic nature of our tagging efforts within the design of a larger sampling program precluded statistically valid sample replication, and only one reserve and adjacent fished area were examined; therefore, the results of this study should not be generalized to other areas. Still, the IRL is typical of other bar-built estuaries where access by estuarine fishes to coastal waters through passes or inlets may be limited, and it is reasonable to expect that the geographical, environmental, and biological processes that influence species movements in the IRL would also be important in other estuaries of similar structure. Studies show that no-take areas in estuarine systems can have an effect on species' abundances and size distributions within these protected areas and may indicate that these areas protect species from the effects of fishing pressure (Johnson et al., 1999; FMRI unpubl. data). Whether or not these areas will actually increase fish abundance in adjacent waters or benefit surrounding fisheries through direct supplemental replenishment of exploitable species is less evident. Certainly, some individuals will migrate out of protected areas in response to environmental, biological, or physiological stimuli, and these individuals may contribute to trophy fisheries in surrounding waters (Roberts et al., 2001); however, our data indicated that within estuaries, reciprocal movements over relatively large distances into protected areas also occur and have the potential to extract exploitable individuals from surrounding fisheries. The overall impact of such withdrawals on these fisheries will depend on the degree of retention following migrations into protected areas. If retention rates are high, then increased egg production, larval export, and juvenile recruitment may be more important mechanisms for replenishment of nearby fisheries than spillover of exploitable species, but production and export will be limited unless reserves encompass spawning or nursery habitats (or both) that will support long-term protection and population growth. For estuarine-dependent coastal species that support estuarine fisheries, the benefits obtained within protected areas will be determined, in part, by their specific life-history characteristics, movement patterns, and the reserve design. Although the establishment and study of reserves in marine or coastal systems has increased in recent years, research on the effects of protected no-take reserves in estuarine habitats is still in its infancy. Information on the daily, seasonal, or annual movement patterns of estuarine-resident or estuarine-dependent coastal species is necessary for understanding and designing effective reserve areas in these habitats.

Acknowledgments

We wish to thank the crewmembers and volunteers at FMRI's Indian River Field Laboratory for collecting data and assisting in this study and the many fishermen who willingly provided us with recapture information. We are grateful to U.S. Fish and Wildlife Service personnel for providing access to sampling areas within restricted areas of the Merritt Island National Wildlife Refuge. This paper benefitted from reviews by R. Cody, J. Colvocoresses, L. French, J. Leiby, R. Paperno, J. Quinn,

T. Tuckey, and F. Vose, and two anonymous reviewers. This work was supported in part by funding from the Department of Interior, U. S. Fish and Wildlife Service, Federal Aid for Sport Fish Restoration Grant Number F-43, and by the State of Florida Recreational Saltwater Fishing License monies.

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Distribution, age, and growth of young-of-the-year greater amberjack (*Seriola dumerili*) associated with pelagic *Sargassum*

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Abstract—Patterns of distribution and growth were examined for young-of-the-year (YOY) greater amberjack (*Seriola dumerili*) associated with pelagic *Sargassum* in the NW Gulf of Mexico. *Seriola dumerili* were collected off Galveston, Texas, from May to July over a two-year period (2000 and 2001) in both inshore (<15 nautical miles [nmi]) and offshore zones (15–70 nmi). Relative abundance of YOY *S. dumerili* (32–210 mm standard length) from purse-seine collections peaked in May and June, and abundance was highest in the offshore zone. Ages of *S. dumerili* ranged from 39 to 150 days and hatching-date analysis indicated that the majority of spawning events occurred from February to April. Average daily growth rates of YOY S. *dumerili* for 2000 and 2001 were 1.65 mm/d and 2.00 mm/d, respectively. Intra-annual differences in growth were observed; the late-season (April) cohort experienced the fastest growth in both years. In addition, growth was significantly higher for *S. dumerili* collected from the offshore zone. Mortality was approximated by using catch-curve analysis, and the predicted instantaneous mortality rate (Z) of YOY *S. dumerili* was 0.0045 (0.45%/d).

Manuscript submitted 9 December 2002 to Scientific Editor's Office.

Manuscript approved for publication 2 March 2004 by the Scientific Editor.

Fish. Bull. 102:545–554 (2004).

Recruitment of marine fishes is highly variable and closely linked to early life events (Houde, 1996; Cole, 1999). Early life survival is dependent upon several biological and environmental factors including spawning time, prey availability, predation pressure, growth, and physical transport mechanisms (Bricelj, 1993; Schnack et al., 1998). Recruitment success is commonly assessed by examining patterns of relative abundance (Sano, 1997), whereas estimates of growth and mortality are commonly used to index recruitment potential (Rilling and Houde, 1999; Rooker et al., 1999). Early life growth and mortality are linked because fishes with high growth rates often exhibit decreased size-specific predator vulnerability (Meekan and Fortier, 1996). As a result, estimates of juvenile abundance, growth, and mortality provide insight into patterns of nursery habitat quality and thus may be used to delineate essential fish habitat (EFH) (Pihl et al., 2000; Sullivan et al., 2000).

Greater amberjack (*Seriola dumerili*) is a reef-associated species with a circumglobal distribution in subtropical and temperate waters (Manooch and Potts, 1997a). In the Gulf of Mexico, *S. dumerili* is the largest carangid and supports important recreational and commercial fisheries (Thompson et al., 1999). Owing to increased fishing effort and landings, *S. dumerili* in the Gulf are currently assessed as overfished (NOAA, 2000). Consequently, detailed life history information is needed to effectively guide fishery management of this valuable resource. To date, available life history data on *S. dumerili* have almost entirely been based on assessments of subadults and adults (Manooch and Potts, 1997a, 1997b; Thompson et al.[1]). Despite the importance of early life processes, data on juvenile or young-of-the-year (YOY) *S. dumerili* are limited to qualitative surveys of pelagic *Sargassum* (Bortone et al., 1977; Settle, 1993).

The National Marine Fisheries Service has recently designated *Sargassum* as essential fish habitat (EFH) of several coastal migratory species including *S. dumerili* (NOAA, 1996). In response, the goal of this study was to examine the distribution and growth of *S. dumerili* associated with pelagic *Sargassum* mats in the NW Gulf of Mexico. Specifically, objectives of this research were to quantify spatial and temporal patterns of habitat use by *S. dumerili* and to determine age, hatching-date, growth, and mortality of *S. dumerili* by using otolith-based techniques.

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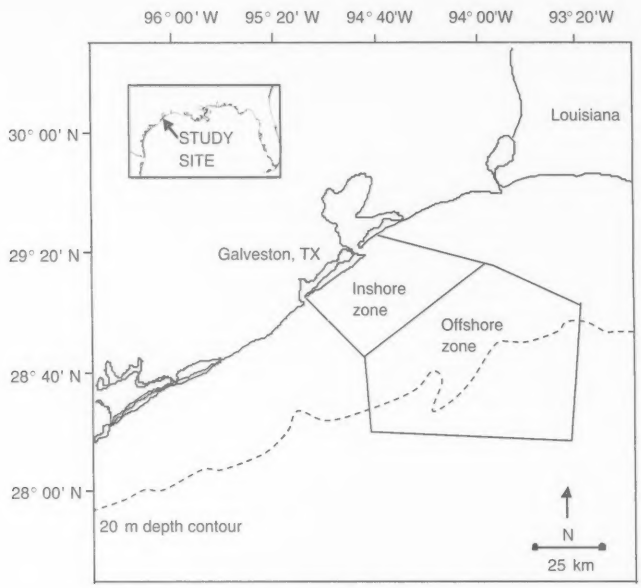

Figure 1

Map of sampling locations along the Texas Gulf coast for *S. dumerili*. Inshore (<15 nautical miles) and offshore (>15 nautical miles) zones off Galveston, TX, are shown.

Materials and methods

Field collections

Seriola dumerili associated with pelagic *Sargassum* mats were collected off Galveston, Texas, from May to July over a two-year period (2000 and 2001) (Fig. 1). Inshore (<15 nautical miles [nmi]) and offshore (15–70 nmi) zones were sampled to evaluate the potential importance of physiochemical conditions because inshore waters off the coast of Texas are heavily influenced by estuarine processes (Smith, 1980; Sahl et al., 1993). Replicate samples (3–5 per trip) in both the inshore and offshore zones were collected monthly by using a larval purse seine (20 m long×3.3 m deep, 1000-μm mesh). The purse seine was deployed into the water as the boat encircled a randomly chosen mat. The seine was pursed, the *Sargassum* was discarded, and fishes were funneled into the codend, collected, and frozen on dry ice. Distribution and abundance were expressed as relative abundance, and catch per unit of effort (CPUE) represented the number of fishes per purse-seine collection. In addition, a small number of YOY *S. dumerili* were collected with hook-and-line for age and growth information only. Standard lengths (SL) were measured to the nearest 0.1 mm, and weights to the nearest 0.1 g before otolith extraction. GPS locations and mat volume (length×width×depth) were recorded at each sample location. Environmental parameters measured included sea surface temperature, salinity, and dissolved oxygen. Daily sea surface temperature data were also taken from NOAA buoy 42035, 22 nmi offshore of Galveston, TX.

Otolith procedures

Sagittal otoliths were extracted from *S. dumerili*. Otoliths were measured to the nearest 0.001 mm and weighed to the nearest 0.0001 g. Left or right sagittae were randomly selected and mounted in epoxy resin (Spurr, 1969). Once mounted, a Buehler isomet low-speed saw equipped with a diamond wafering blade was used to transversely cut embedded otoliths. Otolith sections were then attached to petrographic slides with Crystalbond thermoplastic cement. Type A alumina powder (0.3 μm) and 400- and 600-grit sandpaper were used to grind both sides of the otolith, and a polishing cloth was used for final preparations.

Age was determined by counting growth increments along the sulcus from the core to the outer margin by using a Nikon Labophot-2 light microscope and Optimas 6.2 image analysis software (Media Cybernetics, Silver Spring, MD). Because of the difficulty of enumerating some inner increments near the otolith core, a relationship between age and otolith radius of several clear specimens was used to predict the number of increments within the unclear region. Age was determined by adding the correction factor to the increment count from the first identifiable increment to the otolith margin (Rooker and Holt, 1997). Correction factors consisting of more than five days were applied to 49%

of the fishes and the average correction accounted for 9.5% of the actual age estimate. Otolith readings with correction factors accounting for more than 20% of the predicted age were not used for estimates of growth. The following correction factor was used

$$Age\ (\text{d}) = 2.88 \times otolith\ radius\ (\mu\text{m}) - 0.096 \quad (r^2=0.88,\ n=20).$$

Additionally, all otolith counts were repeated twice to ensure adequate precision. Differences in readings of more than 20% were not incorporated into growth estimates.

Daily deposition of growth increments on sagittal otoliths was validated by using wild *S. dumerili* (n=14, 136–193 mm SL). Fishes caught in the wild were brought into the laboratory and placed in a circular holding tank (1.71 m diameter×0.75 m depth) for 48 hours. Fishes were then placed in a separate tank containing 80 liters of seawater with 100 mg/L of alizarin complexone for two hours (Thomas et al., 1995) and returned to the circular holding tank. Individuals were fed approximately 10% of their body weight daily. Fishes marked with alizarin were removed from the tank after 5 (n=5), 10 (n=5), and 15 (n=4) days. The number of otolith increments between the alizarin mark and outer edge were then counted for daily increment verification. Otolith slides were coded so that all readings were blind.

Hatching dates were determined for all individuals by subtracting daily age from date of capture. An age-specific mortality adjustment was made for individuals because larger *S. dumerili* have spent more time in the early life stages and hence individuals from these cohorts have experienced greater cumulative mortality. Because of the limited number of individuals in 2001, the mortality correction was calculated only for year 2000 collections and applied to hatching-date distributions in 2000 and 2001. Age-specific mortality adjustments were made according to the method described by Rooker and Holt (1997).

Growth and mortality of *S. dumerili* were estimated by using otolith-derived ages. Daily growth rates were estimated by using the linear growth equation

$$SL = slope\ (\text{age}) + y\text{-intercept}$$

and were reported as mm/d. Length-at-age data were also fitted with curvilinear growth models (von Bertalanffy, Laird-Gompertz). Percent variation in length explained by age for both curvilinear models was slightly better at times than the percent variation in length explained by age for the linear model; however, certain model parameters (i.e. L_∞) were biologically unrealistic and thus the linear model was deemed more appropriate. Moreover, when possible, L_∞ values were used to model length-at-age data and the nonlinear models were essentially linear over the limited size range examined. Mortality estimates for year 2000 *S. dumerili* were determined by using a regression on the decline in $\log_e$-transformed abundance on age. A regression coefficient (slope) was used to predict the instantaneous mortality rate:

$$\ln N_t = \ln N_0 - Zt,$$

where N_t = abundance at age t (expressed in days);
N_0 = an estimate of abundance at hatching; and
Z (slope) = the instantaneous mortality coefficient.

Mortality estimates were based upon 10-day cohort groupings. Individuals <40 days old were not included in the mortality regression because of an ascending catch curve and because there were too few individuals >139 days old in our sample—probably owing to gear avoidance or emigration (or both). Therefore, only *S. dumerili* between 40 and 139 days (45–192 mm) were used to estimate mortality.

Data analysis

Effects of location and date on CPUE and size estimates were examined by using a two-way analysis of variance (ANOVA). Levene's test and residual examination established if the homogeneity of variance assumption was met. Normality was evaluated by plotting residuals versus expected values. Abundance data were log (x+1) transformed when necessary to normalize data and reduce heteroscedasticity. Tukey's honestly significant difference (HSD) test was used to determine *a posteriori* differences among means. Comparisons of spatial and temporal variation in growth were performed by using analysis of covariance (ANCOVA). Prior to ANCOVA testing, the homogeneity of slopes assumption was examined using an interaction regression (Ott, 1993). If no significant interaction was detected, ANCOVA models were used to test for differences in length-at-age (y-intercepts) (Ott, 1993). Statistical analysis was carried out by using SYSTAT 8.0 (SYSTAT Software Inc., Richmond, CA), and significance was set at the alpha level of 0.05.

Results

Environmental conditions

Average temperatures from May to July ranged from 27.9 to 30.1°C in 2000 and from 24.5 to 30.4°C in 2001 (Fig. 2). Mean temperatures over the sampling period were 29.2°C and 27.9°C for 2000 and 2001, respectively. Zonal differences occurred: the inshore zone averaged 28.7°C (±0.3) in 2000 and 28.1°C (±0.9) in 2001, and the offshore zone averaged 29.8°C (±0.3) in 2000 and 27.6°C (±0.9) in 2001. Similar to temperature trends, mean salinity was higher in 2000 (34.6‰) than in 2001 (31.9‰) (Fig. 2). Average salinity values gradually increased from an average of 31.5‰ in May to 37.2‰ in July of 2000. A large drop in salinity occurred during

mid-summer of 2001, from 37.6‰ in May to 25.7‰ in June (owing to tropical storm Allison) and rose to 32.3‰ in July. Salinity values were lower and more variable within the inshore zone, ranging from 29‰ to 37‰ (33.4‰ average) in 2000 and from 15‰ to 37‰ (average 28.8‰) in 2001. In contrast, the offshore zone exhibited higher and more stable salinity values, ranging between 33‰ and 38‰ (36‰ average) in 2000, and between 28‰ and 36‰ (34.9‰ average) in 2001. Temperature and salinity values are likely to be influenced by variation in precipitation between years. Precipitation from January through July of 2000 (14.29 inches) was half that of 2001 (29.92 inches) and well below the 30-year average of 22.17 inches (National Weather Service, Dickinson, TX). Dissolved oxygen content was similar between years; values decreased throughout the summer months and were higher within the inshore zone.

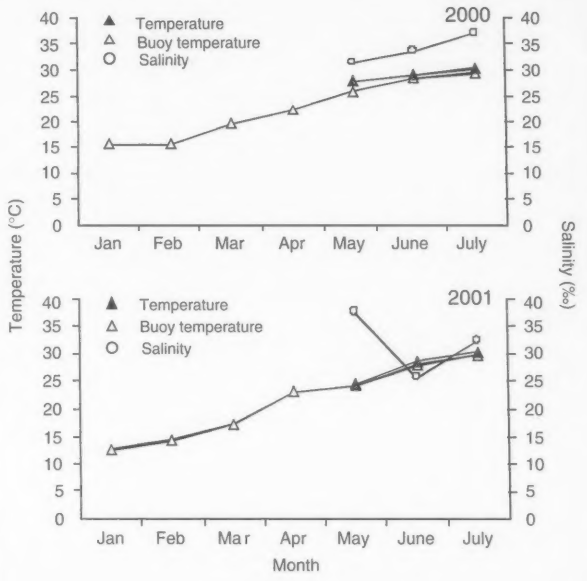

Figure 2

Environmental conditions from January to July of 2000 and 2001. Average temperature (°C) and salinity (‰) values. Open triangles represent temperature data from NOAA buoy 42035, located 22 nautical miles offshore of Galveston, TX.

Spatial and temporal distribution

A total of 181 YOY *S. dumerili* was collected from 42 purse seines over the two-year study period. CPUE values were fourfold higher in 2000 than in 2001, averaging 6.38 (±3.0) and 1.50 (±0.8) per seine, respectively (Fig. 3A). A significant year effect indicated that relative abundance was higher in 2000 (P=0.019). Additionally, CPUE values were higher in the offshore zone in both years (Fig. 3, B and C). However, no significant zonal difference existed in abundance between the inshore and offshore zones in 2000 (P=0.063) or 2001 (P=0.058). Temporal patterns indicated *S. dumerili* was highly abundant in May and June, declining in July in both years (Fig. 3A). A significant seasonal effect occurred for 2000 when highest relative abundance occurred in June with a CPUE of 16.2 (±0.8) (Tukey HSD, P<0.05).

Size comparison

Sizes of *S. dumerili* ranged from 33 to 210 mm SL (mean 125 mm SL ±3.8). Juveniles greater than 100 mm accounted for 68% of the total catch, whereas individuals less than 50 mm accounted for only 15%. Size differences of *S. dumerili* were observed between 2000 (average 125.5 mm) and 2001 (average 141.5 mm); significantly larger *S. dumerili* were collected from the offshore zone in 2001 (P=0.001). A significant interaction (year×month) occurred that indicated that the magnitude of size differences was variable over time. Sizes were also significantly different between zones in 2000; larger individuals were collected within the offshore zone (P=0.025). No zonal comparison was performed for 2001 because few individuals were collected from the inshore zone. In addition, a trend existed within both years: mean sizes significantly increased from May to June, then decreased in July (Tukey HSD, P<0.05).

Hatching-date distribution

Hatching-date distributions for *S. dumerili* were protracted in both 2000 and 2001. Fishes collected in 2000 exhibited hatching-dates from 29 January to 25 May (117 days), whereas those collected in 2001 hatched from 11 January to 30 May (139 days) (Fig. 4). In 2000, over 80% of the fishes appeared to result from spawning events in March and early April. The adjusted distributions from the age-specific mortality correction for both 2000 and 2001 were indistinguishable from those without the correction.

Age and growth

Results of the age-validation exercise indicated that juvenile *S. dumerili* deposit otolith increments on a daily basis (Fig. 5). Average increment counts at day 5, 10, and 15 were 4.8 (±0.2 SD), 9.2 (±0.4), and 14.0 (±0.7), respectively. A relationship between the observed versus expected increments was described by the following equation:

$$\textit{Observed increments} = 0.92\ (\textit{expected increments}) + 0.14 \quad (r^2=0.95)$$

where days after staining represent expected increment count.

Validation of daily growth increments has been observed in a similar study involving juvenile (0–60 days) *Seriola quinqueradiata* (Sakakura and Tsukamoto, 1997).

Age of *S. dumerili* was similar between years; estimated ages ranged from 41 to 150 days (35 to 210 mm SL) in 2000 and from 35 to 120 days (33 to 198 mm SL) in 2001 (Fig. 6). Interannual differences in growth were observed: 2000 (1.65 mm/d), 2001 (2.00 mm/d) (ANCOVA, slope, $P<0.001$) (Fig. 7). A significant cohort effect was also observed; the late-season (April) cohort experienced the fastest growth (ANCOVA, slopes, $P<0.001$) (Fig. 8). Average cohort-specific growth rates of *S. dumerili* spawned in February, March, and April of 2000 were 0.85 mm/d, 1.15 mm/d, and 2.76 mm/d, respectively. In addition, a significant difference in growth was observed for *S. dumerili* collected from inshore (1.55 mm/d) and offshore (1.65 mm/d) zones of 2000 (ANCOVA, slope, $P<0.001$) (Fig. 9). Again, the lack of individuals within the inshore zone in 2001 precluded a comparison between zones for that year.

Mortality

Owing to the limited number of *S. dumerili* collected in 2001, a single catch curve was developed for the 2000 year class, and the mortality coefficient (Z) was 0.0045 (0.45%/d) for individuals between 40 and 139 days (Fig. 10). Cumulative mortality was estimated for the 100-day period (40–139 days), resulting in an overall mortality of 36%.

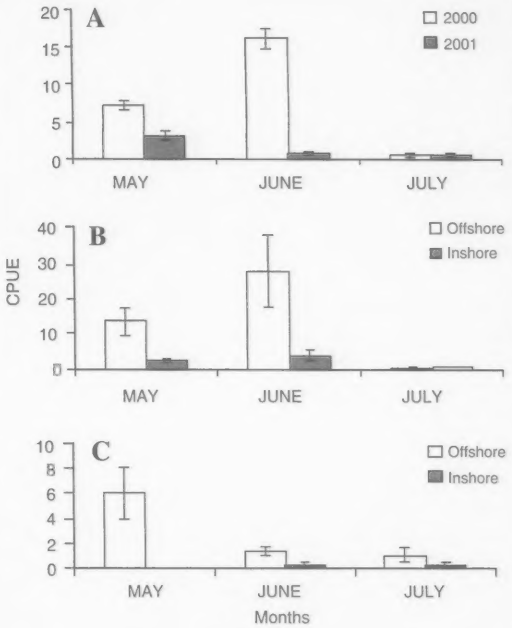

Figure 3

Relative abundance (number per purse seine) (±1 SE) of *S. dumerili* collected in association with *Sargassum* mats: (**A**) 2000 and 2001; (**B**) 2000 by zones; (**C**) 2001 by zones.

Discussion

The size range of *S. dumerili* collected in association with *Sargassum* ranged from approximately 30 to 210 mm (SL), and these sizes are similar to those reported in other studies investigating fish assemblages associated with pelagic *Sargassum*. Bortone et al. (1977) collected several small *S. dumerili* (12–72 mm SL) in the eastern Gulf, whereas individuals collected in the western Atlantic by Dooley (1972) ranged from 13 to 108 mm (SL). Cho et al. (2001) found juvenile *S. dumerili* (35–120 mm TL) associated with drifting *Sargassum* in the western Pacific. Additionally, Sakakura and Tsukamoto (1997) collected over 200 juvenile Japanese amberjack (*S. quinqueradiata*) (18–114 mm TL) associated with pelagic *Sargassum* in the East China Sea. Results of the present study and others indicate that pelagic *Sargassum* mats in the NW Gulf of Mexico serve as nursery habitat for *S. dumerili*.

The limited size range of *S. dumerili* associated with pelagic *Sargassum* indicates that a shift in habitat use may occur at approximately 5–6 months of age. Individuals greater than 210 mm (SL) have not been found in association with pelagic *Sargassum*, and larger *S. dumerili* (ca. 300 mm TL) are relatively common in the recreational headboat fishery in the Gulf of Mexico (Manooch and Potts, 1997a). As a consequence, *S. dumerili* may transition from a pelagic to a demersal existence at the late juvenile stage (between 200 mm SL and 300 mm TL). Pipitone and Andaloro (1995) found a shift in the diet of *S. dumerili*, from a diet predominately consisting of crustaceans toward one of fish >200 mm (SL), further supporting this hypothesis.

Seriola dumerili abundance was greater in the offshore zone than the inshore zone throughout the sampling period. These patterns of habitat use are consistent with earlier information that indicates *S. dumerili* is an offshore species (Hildebrand and Cable, 1930). The proximity to spawning grounds may contribute to the observed spatial patterns because *S. dumerili* are known to spawn in offshore areas (Fahay, 1975). Physiological preferences may also contribute to the dominance of *S. dumerili* in the offshore zone. In our study, salinity values were higher in the offshore zone but more variable within the inshore zone, suggesting that freshwater inflow influences conditions within the inshore zone. Chen et al. (1997) determined that optimum salinity conditions for *S. dumerili* larvae were between 32‰ and 35‰, and larvae remained inactive below a salinity of 30‰. Zonal differences in temperature and dissolved oxygen were also observed. Tzeng et al. (1997)

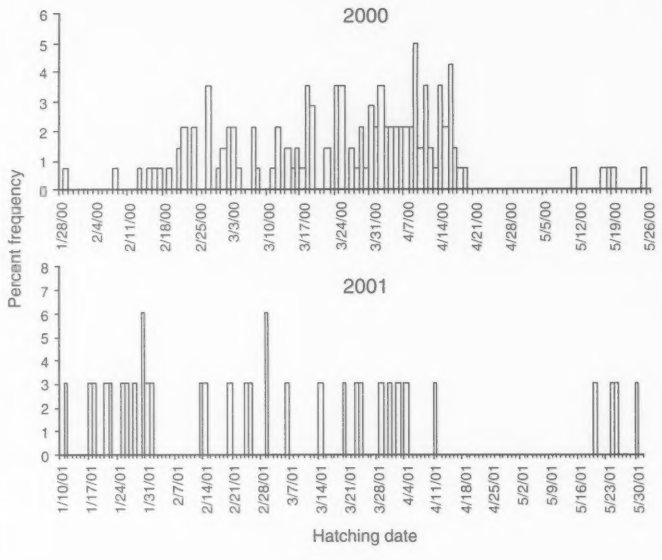

Figure 4

Hatch-date distributions of *S. dumerili* associated with *Sargassum* mats in 2000 and 2001.

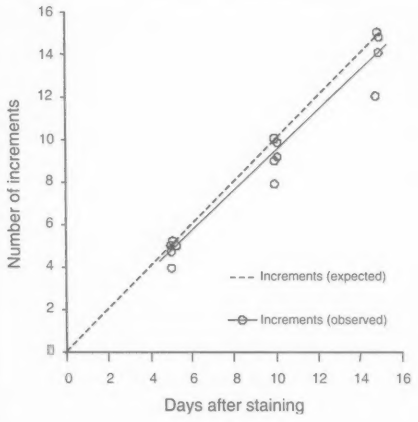

Figure 5

Linear regression of age verification for *S. dumerili*. Circles and solid line represent the number of daily increments observed after staining, and dotted line represents the number of increments expected.

attributed the distribution of fishes from nearshore to offshore stations to environmental factors, season, and life history strategies. Furthermore, the combination of available resources (i.e. food and habitat), seasons, and physiochemical tolerances may account for the observed spatial patterns of habitat use.

Temporal patterns of size-specific habitat use showed similar trends between years and appeared to be related to spawning season. Relative abundance of small *S. dumerili* was highest early in the season (May), declined in June, and further increased late into the season (July) for both 2000 and 2001. Nevertheless, small juveniles were collected during the entire collection period, which suggests that *S. dumerili* spawning in the NW Gulf is protracted. Previous studies have found that *S. dumerili* spawn throughout the spring and summer months (March–July) (Marino et al., 1995; Cummings and McClellan, 1996). In addition, Fahay (1975) suggested, on the basis of larval collections in the western Atlantic, that spawning occurs in the winter. Despite the limited duration of our collection efforts, our results are consistent with these findings with 63% of year-2000 *S. dumerili* and 36% of year-2001 fish resulting from spring spawning events. The remaining individuals were spawned January through early March.

Growth estimates indicated that *S. dumerili* have rapid growth throughout early life stages. Based on linear growth models, average growth of *S. dumerili* was 1.45 mm/d—an estimate similar to that of Manooch and Potts's (1997b) study in the Gulf (average growth of 1.17 mm/d for age-1 individuals). However, growth comparisons may be invalid because their study estimated growth based on counts of annuli and no temperature

data were presented. Because of the lack of studies investigating growth of YOY *S. dumerili*, we compared our estimates to those in Sakakura and Tsukamoto's (1997) study of YOY *S. quinqueradiata* where growth rates were estimated at 1.3 mm/d. Average temperature in their study was 21.2°C, which was considerably lower than the average during our study (28.6°C) and may account for their slower growth rates.

Variation in growth of *S. dumerili* was observed and rates were significantly higher in the offshore zone and greater for the late season cohort. Differences in water temperature may be partly responsible for observed differences in growth. Planes et al. (1999) suggested that spatial differences in growth of juvenile sparid fishes were a result of water temperature and currents. The proximity between zones in this study may have masked differences in hydrography; however, temperatures were higher in the offshore zone (29.8°C, CV=0.03) than in the inshore zone (28.7°C, CV=0.04), and warmer temperatures were likely contributing to faster growth rates in offshore waters. Intra-annual (cohort-specific) growth patterns indicated that the late-season cohort had the fastest growth. Similar to trends between zones, temperature was lowest for the slowest growing cohort (early season) and highest for the fastest growing cohort (late season). Although temperature may affect early life growth of *S. dumerili*, differences in growth may be attributed to other factors such as prey availability and predator activity (Houde, 1987; Paperno et al., 2000; Plaganyi et al., 2000). Moreover, a clear distinction exists in the size classes of YOY *S. dumerili* in comparisons of growth rates and these differences likely contribute to the observed results.

The mortality rate of YOY *S. dumerili* associated with pelagic *Sargassum* was estimated at 0.45 %/d for fishes collected in 2000. These findings are well below similar studies investigating mortality of YOY individuals. Nelson (1998) calculated a mortality estimate of 2.1–2.3%/d for pinfish in three different bay areas in the eastern Gulf of Mexico. In addition, Deegan (1990) estimated YOY menhaden mortality between 1.7 and 2.1%/d in the northern

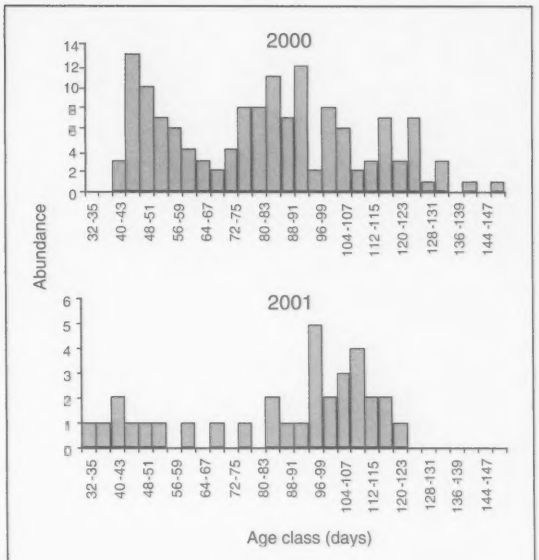

Figure 6

Age-frequency distribution of *S. dumerili* collected in association with *Sargassum* in 2000 and 2001.

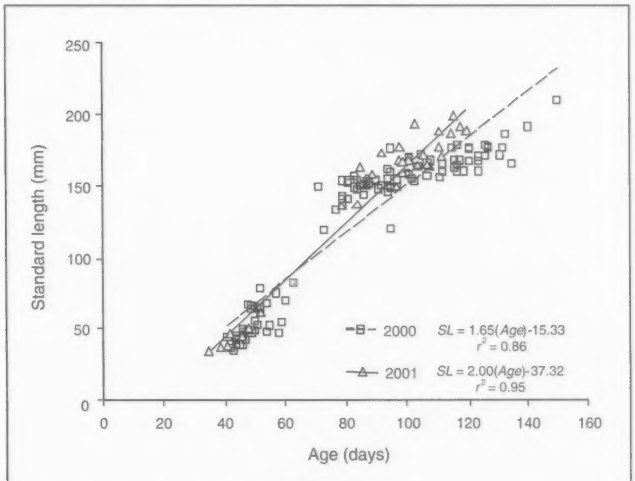

Figure 7

Age-length relationship of *S. dumerili* determined with linear growth curves for interannual comparison.

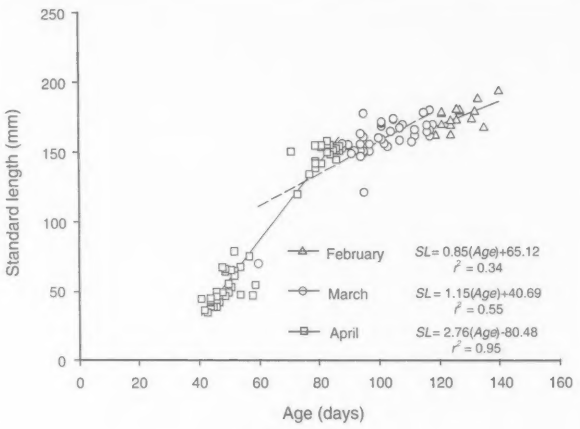

Figure 8

Age-length relationship of *S. dumerili* determined with linear growth curves for a comparison of cohorts of different hatching dates in 2000.

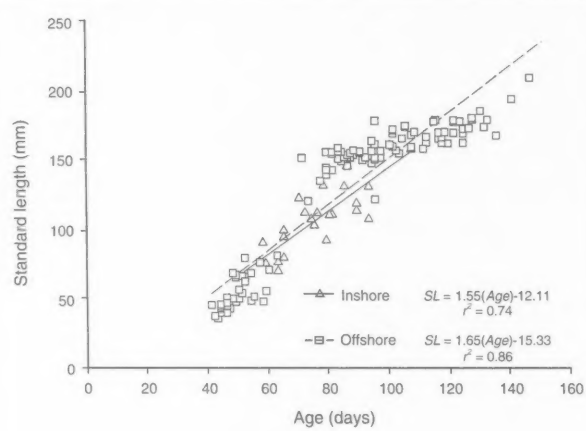

Figure 9

Age-length relationship of *S. dumerili* determined with linear growth curves for a comparison of zones in 2000.

Gulf. These studies included estuarine-dependent species and consisted of smaller individuals. Because our estimates were limited to age 40–139 d individuals, the lack of smaller fishes precluded any mortality estimates of younger *S. dumerili*. These estimates provide baseline information on mortality of YOY *S. dumerili*; however, more detailed studies will be needed to adequately determine mortality rates of YOY *S. dumerili*.

Based on observed patterns of distribution and growth in the NW Gulf of Mexico, early life survival of *S. dumerili* may depend on pelagic *Sargassum*. Results of this study suggest that *S. dumerili* are associated with this habitat over a limited size range and exhibit rapid growth during the first six months. Additionally, *S. dumerili* were more abundant and exhibited higher growth in offshore areas where potential spawning may occur. Thus, *Sargassum* appears to provide nursery habitat for YOY *S. dumerili*, and may influence the recruitment potential of this valuable fishery species.

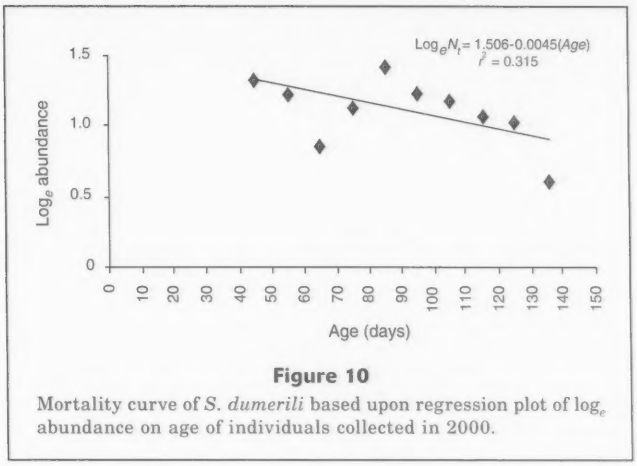

Figure 10

Mortality curve of *S. dumerili* based upon regression plot of $\log_e$ abundance on age of individuals collected in 2000.

Acknowledgments

We thank J. Harper, M. Lowe, B. Geary, J. Turner, and J. Wells for their assistance in the field. Funding for this project was provided by The Aquarium at Moody Gardens (grant 479005 to JRR). Top Hatt charters provided boat time offshore, and Kirk Winemiller and Jaime Alvarado offered constructive criticism and suggestions.

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Identification of formalin-preserved eggs of red sea bream (*Pagrus major*) (Pisces: Sparidae) using monoclonal antibodies

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Catches of important commercial fish such as red sea bream, flat fish, and yellowtail are decreasing in Japan. In order to sustain these species it is especially important that their distribution and biomass at all life stages are known. However, information on the early life stages of these species is limited because identifying the eggs and larvae of such fish is sometimes extremely difficult.

Mito (1960, 1979) and Ikeda and Mito (1988) developed methods for identifying pelagic fish eggs based on morphological features. However, their methods have limitations because many unidentified eggs have similar features. In addition, eggs are usually fixed in formaldehyde solution just after collection in the field. This procedure may alter several egg characteristics and therefore prevent identification (Ikeda and Mito, 1988), or make identification difficult when the egg diameter measures 0.8–1.0 mm because so many kinds of eggs fall in that range. Thus, an alternative identification method would be useful.

Effective genetic analyses for identifying fish eggs or larvae (or both) have been developed by Graves et al. (1989), Daniel and Graves (1994), and Shao et al. (2002). However, their methods may have limitations if samples are preserved in formaldehyde for several years or if DNA must be extracted from numerous samples. In addition, we are lacking the DNA sequences for many species sequences that are necessary for identifying eggs in the field.

We have successfully produced monoclonal antibodies to differentiate harmful marine phytoplankton species from morphologically similar harmless species (Hiroishi et al., 1988; Nagasaki et al., 1991b; Sako et al., 1993; Vrieling et al., 1993; Hiroishi et al., 2002) as well as *Microcystis*, a toxic fresh water bloom-forming cyanobacteria (Kondo et al., 1998). These antibodies were obtained from a culture supernatant solution of hybridoma cells that was produced by a cell fusion procedure between myeloma cells and antibody-producing spleen cells. The specific antibodies described above could be used to detect and quantify harmful bloom-forming microorganisms that react with the monoclonal antibodies and that secondarily react with fluorescein isothiocyanate conjugated goat anti-mouse Ig(G+M) antibody. With fluorescence microscopy with B-exciting light, yellowish fluorescein coronas around the cells of the toxic species were observed, confirming a positive reaction. These antibodies can recognize different molecules distributed on the cell surface, even when the organisms have similar morphological features. One of the molecules distributed on *Chattonella* was determined to be glycoprotein (Nagasaki et al., 1991a). This method would help us to differentiate small marine organisms like fish eggs.

Red sea bream (*Pagrus major*) (Table 1) eggs can easily be distinguished from those of other sparids also found in Japan, such as *Acanthopagrus latus*, by differences in egg size and spawning seasons, and from those of *Evynnis japonica* by differences in spawning seasons (Ikeda and Mito, 1988; Kinoshita, 1988; Hayashi, 2000). However, eggs of some sparids, such as *Acanthopagrus schlegeli*, *Sparus sarba*, and *Dentex tumifrons* are extremely difficult to distinguish from eggs of *P. major*. Therefore, we developed monoclonal antibodies that allow *P. major* eggs to be clearly identified by immunostaining, thus differentiating them from other similar sparids.

This technique may be a useful new tool for identifying fish eggs. Here, we report a method for identifying *P. major* eggs using monoclonal antibodies developed to react specifically with the eggs.

Materials and methods

Eggs of *P. major* were obtained from adult female fish that had spawned in

Manuscript submitted 4 April 2003
to Scientific Editor's Office.
Manuscript approved for publication
2 March 2004 by the Scientific Editor.
Fish. Bull. 102:555–560 (2004).

Table 1
Characteristics of Sparidae distributed in Japan.

Suborder	Species	Distribution	Spawning season	Egg size (mm)	Egg oil globule size (mm)
Pagrinae	*Pagrus major*	South of Hokkaido (Coastal)	Mar–May	0.90–1.03	0.19–0.25
	Evynnis japponica	South of Hokkaido (Coastal)	Oct–Dec	0.89–0.98	0.19–0.21
Sparinae	*Acanthopagrus schlegeli*	South of Hokkaido (Coastal)	Mar–Jun	0.83–0.91	0.20–0.22
	Acanthopagrus latus	South Japan (Coastal)	Oct–Nov	0.76–0.81	0.2
	Sparus sarba	South Japan (Coastal)	Apr–Jun	0.88–0.92	0.19–0.22
Denticinae	*Dentex tumifrons*	South Japan (Oceanic)	May–Jun	0.90–0.93	0.19

isolation tanks at several sea farming centers described in Table 2. Immediately after collection, fish eggs were fixed in a solution of 5% formaldehyde to sea water solution and stored. Before use, the eggs were thoroughly washed with distilled water and suspended in phosphate buffered saline (PBS) solution.

Monoclonal antibodies were developed according to the methods of Köhler and Milstein (1975), Garfré and Milstein (1981), and Hiroishi et al. (1984, 1988): 0.5 mL of egg suspension (200 eggs/PBS solution from Fukui Prefectural Sea Farming Center, Obama City, Fukui Prefecture) was mixed with 0.5 mL Freund's complete adjuvant (Nacalai Tesque, Inc., Kyoto, Japan). The mixture were then injected subcutaneously into BALB/c female mice (4 weeks of age). The female mice received second and third injections at 2-week intervals. For the final immunization, *P. major* eggs collected in the sea farming center of Kansai Environmental Engineering Center Co. (Miyazu City, Kyoto, Japan) were injected into the mouse after being emulsified with Freund's incomplete adjuvant (Nacalai Tesque, Inc.). Three days after the final immunization, the spleens of the mice were removed and passed through a mesh (mesh size: 100 μm). The spleen cells obtained by this procedure were fused with the myeloma cell line X63-AG8.653 at a ratio of 10:1 with 50% polyethylene glycol. After cell fusion, hybrid cells were incubated in a selective hypoxanthine-aminopterin-thymidine medium (Köhler, 1979; Garfré and Milstein, 1981).

The reactivity of the antibodies produced by the hybridomas was then determined. Eggs fixed with 5% formaldehyde in seawater were washed with PBS solution in a 96-well plate. Throughout the experiments, the principal eggs used were from the Fukui Prefectural Sea Farming Center. Normal horse serum solution (200 μL), diluted 100-fold with PBS, was added to the wells to prevent any nonspecific reactions. After incubation at room temperature for 20 minutes, the eggs were washed with 200 μL of PBS. After removing the PBS, 200 μL of the hybridoma culture supernatant solution was added to the wells and incubated at room temperature for 30 minutes. After washing with PBS (100 μL), biotinylated horse anti-mouse IgG (100 μL) was added to the wells and incubated at room temperature for 20 minutes. After the incubation, VECTASTAIN^{R} ABC reagent (avidin DH + biotinylated horseradish peroxidase/PBS, 100 μL) was added according to the direction of VECTASTAIN^{R} Elite ABC kit (ABC Mouse IgG Kit, Funakoshi Co., Tokyo, Japan). After immunostaining the eggs were observed by stereoscopic microscopy (SMZ-2T, Nikon Co., Tokyo, Japan). In a positive reaction, the surface of the fish egg was stained brown as a result of the oxidation of 3,3′-diaminobenzidine (substrate) by horseradish peroxidase bound to the egg surface by the antibody.

Unidentified pelagic fish eggs from open water were collected by using a plankton net (MTD net, NGG54 with mesh size of 0.344 mm, Rigo Co., Tokyo, Japan) from Wakasa Bay (Fukui Prefecture, Japan) in May 1997. They were fixed with 5% formaldehyde in sea water, either immediately or after incubation in seawater in finger bowls at 20°C for 24 hours, and identified by careful observation as described by Ikeda and Mito (1988) and Ikeda et al. (1991). The fixed eggs were transferred to net wells (mesh size 200 μm, diameter 24 mm, Corning Incorporated, Corning, NY) and washed with 10 mL of distilled water three times. Then the eggs in the netwells were immersed in 100 mL of PBS in a polystyrene tray (Corning Incorporated, Corning, NY) for 5 minutes. The egg suspension was placed into the wells of a six-well plate and incubated with 10 mL of normal horse serum solution for 20 minutes. After incubation, the eggs were incubated with 10 mL of MT-1 antibody solution (hybridoma culture supernatant) and then incubated with 10 mL of biotinylated horse anti-mouse IgG. The subsequent procedure was performed as described above.

The immunoglobulin subclass of monoclonal antibodies was determined according to the directions of the mouse monoclonal antibody isotyping kit (Amersham Pharmacia Biotech Co., Uppsala, Sweden) as follows: 3 mL of monoclonal antibodies solution (hybridoma supernatant solution) obtained in this study was added to 0.3 mL of horseradish peroxidase-conjugated anti-mouse IgG in the kit. An isotyping stick in the kit was incubated with the above solution at room tem-

perature for 15 minutes. Then the stick was washed with 0.1% Tween 20/PBS, and incubated with 4-chloro-1-naphthol solution (substrate of horseradish peroxidase in the kit) containing 0.1% H_2O_2 at room temperature for 15 minutes. The immunoglobulin subclass of the monoclonal antibodies was determined by observing the positions of bands that appeared on the stick.

Results and discussion

After cell fusion, hybridomas were grown in 42 wells of 96-well plates. Supernatant solutions of the cultures were used for the immunostaining assay to select hybridomas producing antibodies reactive to *P. major* eggs. After the assay, positive reactions were observed in six wells. These hybridomas were cloned by the limiting dilution method, and finally three clones producing monoclonal antibodies reactive with *P. major* were obtained. Those antibodies were named MT-1, MT-2, and MT-3. The subclass of all antibodies was IgG_1. Specificity of the antibodies was examined by using the eggs shown in Table 2. As a result, the antibodies were reactive with all the *P. major* eggs in both the early and late stages (before or after tail-bud stage), but not with eggs of other species (Table 3, Fig. 1). Thus, it becomes possible to identify *P. major* eggs. The immunostaining assay took 2.5 hours.

The oldest eggs of *P. major* (20 April, 1995) could react with the antibodies obtained as clearly as the recently collected eggs of *P. major*, indicating that egg samples preserved for up to 7 years could be analyzed by this method.

The method was also successful with 102 eggs collected from Wakasa Bay (Table 4), which had been immediately fixed with 5% formaldehyde in seawater. Among them, only 11 eggs were identified as *Callionymoidei* spp.

Table 2
Fish eggs used in the experiment

Egg no.	Species	Sampling location	Stage of eggs	Sampling day
1	*Pagrus major*	Kansai Environmental Engineering Center (Miyazu, Kyoto Pref)	Early	20 Apr '95
2		Kansai Environmental Engineering Center (Miyazu, Kyoto Pref.)	Late	21 Apr '95
3		Fukui Prefectural Sea Farming Center (Obama, Fukui Pref.)	Early	2 Jun '95
4		Fukui Prefectural Sea Farming Center (Obama, Fukui Pref.)	Late	2 Jun '95
5		Kyoto Prefectural Sea Farming Center (Miyazu, Kyoto Pref.)	Early	31 May '96
6		Kyoto Prefectural Sea Farming Center (Miyazu, Kyoto Pref.)	Early	31 May '96
7		Faculty of Agriculture, Kyushu University (Fukuoka, Fukuoka Pref.)	Early	6 Jun '96
8		Fukui Prefectural Sea Farming Center (Obama, Fukui Pref.)	Early and late	21 May '97
9		Osaka Prefectural Fisheries Station and Sea Farming Center (Osaka Pref.)	Early	11 May '95
10		Osaka Prefectural Fisheries Station and Sea Farming Center (Osaka Pref.)	Late	19 May '95
11	*Acanthopagrus schlegeli*	Faculty of Agriculture, Kyushu University (Fukuoka, Fukuoka Pref.)	Early	6 Jun '96
12		Osaka Prefectural Fisheries Station and Sea Farming Center (Osaka Pref.)	Early	19 May '97
13		Osaka Prefectural Fisheries Station and Sea Farming Center (Osaka Pref.)	Late	19 May '97
14	*Acanthopagrus latus*	Himedo, Amakusa, Kumamoto (Kumamoto Pref.)	Unknown	21 Nov '98
15	*Sparus sarba*	Faculty of Agriculture, Kyushu University (Fukuoka, Fukuoka Pref.)	Late	3–5 Jun '96
16	*Dentex tumifrons*	Ohara, Chiba (Chiba Pref.)	Unknown	Oct '98
17		Japan Sea-Farming Association (Miyazu, Kyoto Pref.)	Early	20 Apr '95
18	*Paralichthys olivaceus*	Japan Sea-Farming Association (Miyazu, Kyoto Pref.)	Late	21 May '97
19		Fisheries Laboratory of Kinki University (Kushimoto, Wakayama Pref.)	Late	15 Jan '97
20		Japan Sea-Farming Association (Miyazu, Kyoto Pref.)	Early and late	23 Apr '97
21	*Engraulis japonica*	Wakasa Bay (Fukui Pref.)	Unknown	May '97

(type II). The remaining 91 unidentified eggs were divided into three groups (types I, III, and IV) based on diameters. Of these 91, 51 type-II eggs reacted with MT-1. This finding is compatible with the possibility that the eggs were *P. major*, because the size was similar to that of *P. major* and each contained a single oil globule of a similar size (Tables 1 and 4). Another 43 eggs were collected from another area of Wakasa Bay (Table 5). None of the eggs fixed just after collection were morphologically identifiable. But, after incubation at 20°C for 24 hours until the late stage, all six eggs identified as *P. major* were reactive with the antibody MT-1, whereas the others were not. These findings strongly suggest that the method developed in this study is useful for identifying *P. major* eggs in seawater. Although only late stage eggs were used in this experiment, early stage eggs are also detectable because the antibody recognized both stages of *P. major* eggs from several sea farming centers (Table 2).

Compared to genetic analysis of fish eggs, this method has the advantage of being able to assay many eggs simultaneously without the need to separate individual eggs in tubes and without extracting DNA from the individual egg in each tube. Further, this method works with formalin-fixed eggs, whereas extraction of DNA from formalin-fixed material is problematic. Plankton samples from field studies are typically fixed in formalin-seawater solution.

There was no problem obtaining a large amount of the monoclonal antibody required when identifying *P. major* eggs. The antibody can be easily obtained by large-scale cultures of hybridoma cells. About 50 mL of antibody solution was obtained after two weeks of cultivation. There was no technical problem assaying 43 or 102 eggs from natural waters. However, one assay of a field sample cost about 20 U.S. dollars. To keep costs down an assay kit cheaper than the VECTASTAIN^{R} Elite ABC kit is needed when a large number of field samples are analyzed.

Table 3

Reactivity of monoclonal antibodies to fish eggs. + represents positive reaction; – represents negative reaction.

		Reactivity		
Egg no.	Species	MT-1	MT-2	MT-3
1	*Pagrus major*	+	+	+
2		+	+	+
3		+	+	+
4		+	+	+
5		+	+	+
6		+	+	+
7		+	+	+
8		+	+	+
9	*Acanthopagrus schlegeli*	–	–	–
10		–	–	–
11		–	–	–
12		–	–	–
13		–	–	–
14	*Acanthopagrus latus*	–	–	–
15	*Sparus sarba*	–	–	–
16	*Dentex tumifrons*	–	–	–
17	*Paralichthys olivaceus*	–	–	–
18		–	–	–
19		–	–	–
20		–	–	–
21	*Engraulis japonica*	–	–	–

Acknowledgments

We would like to thank the following sea farming centers and universities for providing the fish eggs used in this study: Fukui Prefectural Sea Farming Center; Kyoto Prefectural Sea Farming Center; Faculty of Agriculture, Kyushu University; Osaka Prefectural Fisheries Station; Sea Farming Center of the Japan Sea-Farming Association; Fisheries Laboratory of Kinki University. We also thank Jeffrey M. Leis, Australian Museum, Sydney, Australia, for his kind advice during the writing of this manuscript.

Table 4

Reactivity of monoclonal antibody MT-1 to the pelagic eggs fixed with formaldehyde just after collection from Wakasa Bay. O.G. diameter = oil globule diameter

Fish egg type	Egg diameter (mm)	O.G. diameter (mm)	Reactivity (%) (positive egg no./ total egg no.)
I	0.72–0.79	0.16-0.19	0 (0/2)
II	0.75–0.82	no oil globule	0 (0/11)
III	0.81–1.02	0.19–0.28	58 (51/88)
IV	1.07	0.21	0 (0/1)

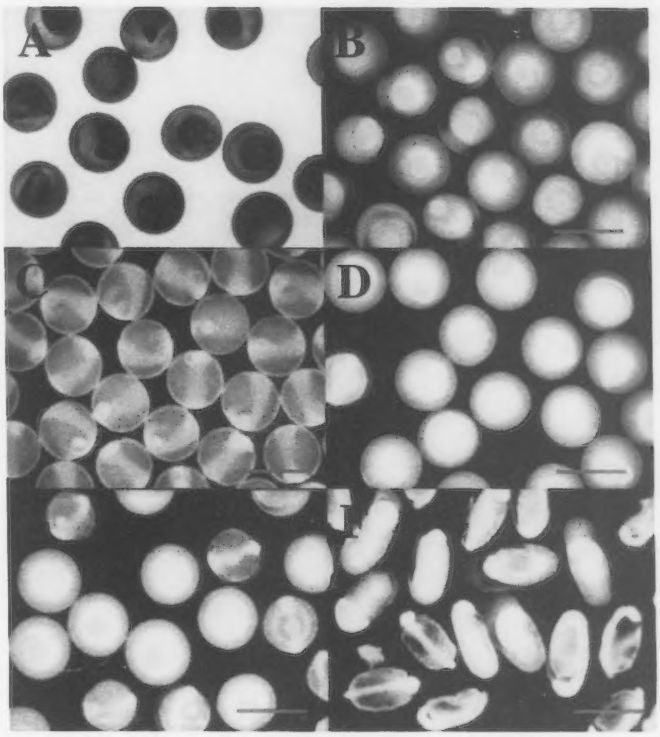

Figure 1

Reactivity of monoclonal antibody MT-1 to fish eggs detected by immunostaining. (**A**) *Pagrus major* (positive reaction); (**B**) *Pagrus major* (negative control reaction without primary antibody); (**C**) *Acanthopagrus shlegeli*; (**D**) *Sparus sarba*; (**E**) *Paralichthys olivaceus*; (**F**) *Engraulis japonica*. Bar represents 1 mm. Only the *P. major* eggs stained brown and showed a positive reaction.

Table 5

Reactivity of monoclonal antibody MT-1 to the pelagic eggs reared for 24 hours after collection from Wakasa Bay.

Egg	Species	Reactivity (%) (positive egg no./total egg no.)
Fish	*Pagrus major*	100 (6/6)
	Acanthopagrus shlegeli	0 (0/8)
	Paralichthys olivaceus	0 (0/1)
	Triglidae sp.	0 (0/1)
	Konosirus punctutus	0 (0/2)
	Soleoidei sp.	0 (0/7)
	Englauris japonicus	0 (0/13)
Decapod	*Enploteuthidae sp.*	0 (0/5)

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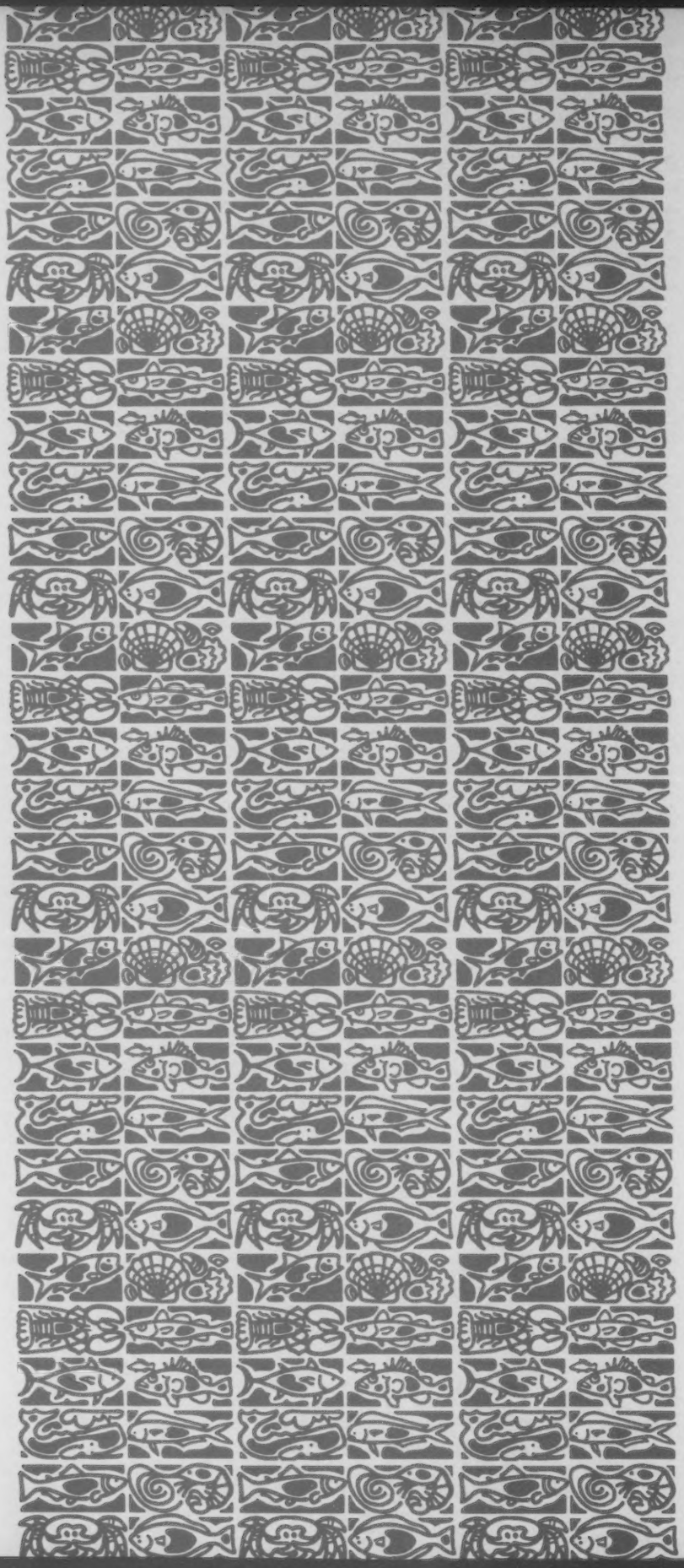
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